

Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

a50298
.A105
no. 49

See

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19-21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

PROCEEDINGS

USDA
NAT'L AGRIC LIBRARY
2002 DEC 20 P 11:37
CONG. SERIALS RECD.
SERIALS BRANCH



49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Executive Committee:

General Chairperson:

Robert C. Edmondson, Applied Engineering & Science

Technical Chairperson:

Rodney Frazier, Frazier Barnes and Associates

Technical Co-Chairperson:

Mike Erickson, AC Humko

Poster Session Chairperson:

Michael K. Dowd, SRRC/ARS/USDA

Tabletop Exhibit Chairperson:

Dick Gadomski, PSI Process Systems, Inc.

Organizing Committee:

Richard Barton, N. Hunt Moore and Associates, Inc.

Philip A. Bollheimer, Bollheimer & Associates Inc.

Michael J. Boyer, Applied Engineering & Science

Donald E. Britton, Mid-Continent Labs

John P. Cherry, USDA, ERRC

Steve Doty, Alternative Sources

Walter Farr, De Smet Process & Technology

Lynn A. Jones, National Cottonseed Products Association, Inc.

J. Patrick Jordan, USDA, SRRC

Juan Kindelan, Protein Technologies International

Tristan Merediz, PSI Process Systems, Inc.

Jeffrey L. Newman, AOCS

Stan Smith, Retired

Robert L. Stroup, The R.L. Stroup Company Ltd.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

PROGRAM SCHEDULE



49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

IMPORTANT NOTICE

Preprints of papers are distributed at this meeting for the personal use of registrants only. Persons who wish to reproduce or to publish a paper must contact the author(s) for permission. U.S. copyright law specifies that copyright is vested in the individual who writes the paper or who paid to have the paper written. Papers prepared by federal employees as part of their jobs may not be copyrighted.

The papers in these proceedings have been reproduced exactly as submitted by the authors.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

PROGRAM SCHEDULE

Sunday, March 19, 2000

2:00 p.m.–7:00 p.m.

1:00 p.m.–5:00 p.m.

6:00 p.m.–7:00 p.m.

Registration

Table Top Exhibit Set Up

Opening Reception

Foyer: Crescent Ballroom

International Ballroom

International Ballroom

Monday, March 20, 2000

7:30 a.m.–5:00 p.m.

7:30 a.m.–8:30 a.m.

8:30 a.m.–9:30 a.m.

Registration

Continental Breakfast

Opening Remarks

Foyer: Crescent Ballroom

International Ballroom

Crescent Ballroom

INVOCATION

David H. Kinard

National Cottonseed Products Association, Memphis, TN

CALL TO ORDER BY GENERAL CHAIRPERSON

Robert C. Edmonson, Senior Vice President

Applied Engineering & Science, Big Sandy, TX

KEYNOTE PRESENTATION:

An Outlook for the U.S. Oilseeds Industry

John C. Baize, John C. Baize & Associates, Falls Church, VA

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

SESSION I

Trade, Marketing, and Economics

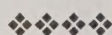
Monday, March 20, 2000

9:30 a.m. – 12:00 Noon

Crescent Ballroom

Session Chairperson: Lynn Jones, The National Cottonseed Products Association, Inc.,
Memphis, TN

- A. **Thriving in a Consolidating Marketplace.**
David Bossman, American Feed Industry Association, Arlington, VA
- B. **Modifying a Commodity Crop to Survive in the New Millennium—the Sunflower and Soybean Experience.**
Edward J. Campbell, Archer Daniels Midland Company, Decatur, IL
- C. **GM Crops: Current Status and Future Prospects.**
Dwayne Buxton, Agricultural Research Service, USDA, Beltsville, MD
- D. **Washington Perspective—Regulatory, Environmental, *Trans* Labeling.**
Robert Reeves, Institute of Shortening & Edible Oils, Washington, DC



LUNCHEON

Monday, March 20, 2000

12:00 p.m.–1:45 p.m.

International Ballroom

dedicated time to view poster presentations and visit with table top exhibitors

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

SESSION II

Science and Technology

Monday, March 20, 2000

2:00 p.m.–5:00 p.m.

Crescent Ballroom

Session Chairperson: John P. Cherry, Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA

- E. **ARS Research Programs in Oilseeds Quality and New Uses.**
Frank Flora, National Program Staff, Agricultural Research Service, USDA, Beltsville, MD

- F. **Specialty Oils—Characteristics and Concerns.**
Kathleen Warner, Food Quality and Safety Research, USDA, NCAUR, Peoria, IL

- G. **Research in Alternative Solvents.**
Peter J. Wan, Southern Regional Research Center, ARS, USDA, New Orleans, LA

- H. **Current States of Membrane Technology in Oilseed Processing and Edible Oil Refining.**
S. Sefa Koseoglu, Texas A&M University, College Station, TX

- I. **Strategic Plans for Federal Role in Advancing Biobased Products.**
Howard N. Rosen, USDA Forest Service, Washington, DC

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Tuesday, March 2, 2000

7:30 a.m.–2:00 p.m. Registration

Foyer: Crescent Ballroom

8:00 a.m.–9:00 a.m. Continental Breakfast

International Ballroom

SESSION III

Management

Tuesday, March 21, 2000

9:00 a.m.–12:00 noon

Crescent Ballroom

Session Chairperson: Michael J. Boyer, Applied Engineering & Science, Atlanta, GA

- J. **Down the Road to Value Added.**
Wayne Martin, PYCO Industries, Lubbock, TX
- K. **Overview of EPA Vegetable Oil MACT Rule—Update on Rule Provisions.**
Robert L. Ajax, Robert L. Ajax & Associates, Apex, NC
- L. **Specialty Oils Outlook—New Realities.**
Bill Soucie, Protein Technologies International, St. Louis, MO
- M. **Leadership and the Changing Work Environment.**
Joan L. Bicocchi, JB Consulting, New Orleans, LA



LUNCHEON

Tuesday, March 2, 2000

12:00 p.m.–1:45 p.m.

International Ballroom

Featured Speaker: **Rod Smith**, Feedstuffs Newspaper, Minnetonka, MN

"Consolidation to Build Branded, Global House"

GRAND PRIZE DRAWING AT LUNCHEON - Shotgun w/case

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

POSTER SESSION



M

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

POSTER SESSION

Sunday, March 19, 2000 - 6:00 p.m.–7:00 p.m.

Monday, March 20, 2000 - 12:30 p.m.–1:30 p.m.

Session Chairperson: Michael K. Dowd
USDA, ARS, Southern Regional Research Center
New Orleans, LA

Posters will be on display throughout the conference.
Be sure to visit with the poster presenters at the times noted above.

Effects of Temperature on Production of Polyunsaturated Fatty Acids in Yeast Expressing a Plant Enzyme. John M. Dyer, Dorselyn C. Chapital, and Armand B. Pepperman, USDA-ARS-SRRC, New Orleans, LA

Formulation, Structure and Properties of Commercial Spreads, a 1999 Survey. G.R. List, K.R. Steidley, and W.E. Neff, Agricultural Research Service, USDA, Peoria, IL

Technologies Supporting the Adoption of Biodiesel as an Alternative Fuel. Thomas A. Foglia*, Kerby C. Jones, Michael J. Haas, and Karen M. Scott, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA

Identification of Genes for Enzymes Involved in the Synthesis of Tung Oil. H. Shepherd¹, J. Dyer¹, F. Tang², D. Chapital¹, D. Shih², and A. Pepperman¹, ¹Southern Regional Research Center, ARS, USDA New Orleans, LA; and ²Department of Biological Sciences, LSU, Baton Rouge, LA

Gossypol Isomers in Seed of Upland (*Gossypium hirsutum*) and Pima (*Gossypium barbadense*) Cottons. M.C. Calhoun, B.C. Baldwin, and S.W. Kuhlmann, Texas Agricultural Experiment Station, The Texas A & M University System, San Angelo, TX

Early Harvest Can Reduce Aflatoxin Contamination of Cottonseed. C. H. Bock and P. J. Cotty, Research Plant Pathologists, Food and Feed Safety Research, Southern Regional Research Center, ARS, United States Department of Agriculture, New Orleans, LA

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Domestic Production of Castor Oil. Thomas A. McKeon, Q. Grace Chen, Karen M. Lew, Allan E. Stafford, and Jiann-Tsyh Lin, USDA-ARS Western Regional Research Center Albany, CA

Simple Method for the Determination of Free Fatty Acid Content in the Oil of Fuzzy Cottonseed. Peter J. Wan¹, David R. Pakarinen¹ and D. W. Bell², ¹Southern Regional Research Center, ARS, USDA New Orleans, Louisiana; ²Chickasha of Georgia, Tifton, Georgia

Bioconversion of Fats and Oils into Value-Added Products. William N. Marmer¹, Thomas A. Foglia, Daniel K.Y. Solaiman, and John P. Cherry, USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19-21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

TABLE TOP EXHIBITS



49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Table Top Exhibits

Alfa Laval, 200 South Park Blvd., Greenwood, IN 46143 USA. Alfa Laval is a supplier of process equipment for the edible oil industry. Products include high-speed separators, decanters, mixers, SoftColumn deodorizers, and heat exchangers. Alfa Laval has recently introduced a new high-capacity PX-100 separator. Separators are available for all edible oil processes. Alfa Laval supplies complete processes, including miscella refining, degumming, neutralization, dewaxing, and deodorization.

AOCS, P.O. Box 3489, Champaign, IL 61826-3489 USA. AOCS Press will display industry-related books and new releases. Among the titles on display will be: *Recent Developments in the Synthesis of Fatty Acid Derivatives*, *Bleaching and Purifying Fats and Oils: Theory and Practice*, *Handbook of Fats and Oils Technology*, and *Technology and Solvents for Extracting Nonpetroleum Oils*. And, you also will find AOCS Membership, Division, and Technical information.

Buhler Inc., P.O. Box 9497, Minneapolis, MN 55440-9497, 100 Xenium Lane, Plymouth, MN 55441 USA. Representatives will be on hand to discuss Buhler's complete range of oilseed processing equipment and turnkey plant capabilities. Buhler manufactures preparation, meal grinding, cleaning, unloading and storage equipment.

Central Hanse Analytical Laboratory, LLC, 101 Woodland Highway, Belle Chase, LA 70037 USA. Central Hanse Analytical Laboratory is a commercial testing laboratory offering GMO testing by PCR and by ELISA. The lab also has 30 years of experience providing chemical and microbiological testing with extensive experience in vegetable oil analysis. Through our inspection arm, we can sample shipments at major ports and offer IP certificates. Visit our Web site: www.gmotesting.com.

Crown Iron Works Company, P.O. Box 1364, Minneapolis, MN 55440 USA. The Crown Group provides complete design and supply services for oilseed and edible oil processing. Crown's Oilseed Division specializes in solvent extraction, refining, methyl ester and oleochemical technology. Crown has offices in England, Brazil, Honduras, Moscow, and China, to service its worldwide customer base.

De Smet Process & Technology, 450 Franklin Road, Suite 160, Marietta, GA 30067 USA. De Smet will have technical experts to discuss its full range of process equipment and services, including mechanical and solvent extraction, oil processing, and fat modification. De Smet will also introduce the new technologies recently purchased from French Oil Mill Machinery Company. All of the partners of the Alliance (Kice, Rotex, Roskamp/Champion) will be represented.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Divine Engineering, Inc., 5440 6th Street SW, Cedar Rapids, IA 52404 USA. Divine Engineering, Inc., manufactures rugged, heavy-duty, bulk-handling chain and flight drag conveyors, known as Divinalators and Divinaflows. Divine is known throughout the grain processing industry for high quality engineering and workmanship, especially on vapor-tight units. The success of its machines is due to simplicity of design, use of standard, high-quality components, and exceptional craftsmanship. Heavy duty machine construction pays off in years of trouble-free service. Divine equipment, engineered and manufactured in Iowa, has been proven in the field since 1948.

The Essmueller Company, P.O. Box 1966, Laurel, MS 39441 USA. Founded in 1878, The Essmueller Company is a leading manufacturer of drag conveyors and bucket elevators for industrial processing industries. Essmueller is now building screw feeders and introducing its new and innovative V-Model enclosed belt conveyor for heavy duty, high capacity grain handling applications.

Grain Systems Inc. (GSI), 1004 East Illinois Street, P.O. Box 20, Assumption, IL 62510. GSI is a global manufacturer of products for the agricultural community. GSI owns and operates ten warehouses, seven manufacturing facilities in the US, and 3 outside the US. GSI manufactures corrugated storage vessels, bucket elevators and chain conveyors, sweep augers, aeration systems, scalpings and cleaners, process and grain dryers, and complete systems for poultry and swine.

Hi Roller Conveyors, 5100 West 12th Street, Sioux Falls, SD 57107-0514 USA. Hi Roller manufactures enclosed belt conveyors that automatically reload dust or spilled material. Products include the Hi Roller conveyor and the Consignor enclosed moveable tripper.

I.C. Thomasson Associates Inc. / Stanley Jones Corporation, Inc., 2950 Kraft Drive, Suite 500, Nashville, TN 37204 USA. I.C. Thomasson and Stanley Jones Corporation have a quarter century of joint design/build experience in process and power related projects. Both firms, established in the 1940's, rely on quality in design and construction, to keep clients returning, project after project.

Industrial Filter & Pump Manufacturing Company, 5900 W. Ogden Avenue, Cicero, IL 60650. USA. Industrial Filter & Pump will feature filtration equipment for oil processing (crude and refining) featuring Type 122 Horizontal Tank, Vertical Leaf Filters from 50 to 2000 ft². Type 112 Vertical Tank, Vertical Leaf filters will also be displayed, along with filter presses.

InterSystems, Inc., 13330 'I' Street, Omaha, NE 68137 USA.. InterSystems manufactures en-masse conveyors, enclosed belts, bucket elevators, automatic bulk weighing systems, automatic sampling, and automatic probes.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Laidig Industrial Systems, 14535 Dragoon Trail, Mishawaka, IN 46544-6896 USA. Laidig's new Silo Unloader - Model 4345 is the solution to your silo/bin bridging problems. Laidig Industrial Systems introduces the new improved cone bottom Model 4345. This cone bottom unloader has a sweep auger rotating 360° around the bin. This helps create mass flow, and enhances a first-in, first-out (FIFO) flow. Model 4345 handles gray seed, white seed, black seed, hulls and lint, meals, and many more materials. All material contact surfaces can be manufactured in stainless steel. In addition, to the complete line of cone bottom models, Laidig offers an entire family of heavy-duty, flat-bottom unloaders. Laidig silo unloaders can be installed into a new or existing silo/bin. Call Laidig for an answer to your material handling need, and visit our Web site: www.laidig.com.

L.F.C. North America, 20000 Governors Drive, Olympia Fields, IL 60461 USA. On display will be L.F.C. North America filtration equipment for all processing steps in edible/vegetable oil refining. L.F.C. North America features a 5-ply filter leaf design and has the ability to re-screen or provide new filter leaves for almost any make of filter.

Lurgi PSI Inc., 1790 Kirby Parkway, Ste. 300, Memphis, TN 38138 USA. Lurgi representatives will present their experience in design, engineering, and construction of state-of-the-art processing units for oil seed extraction, oil refining, and modification for oleochemicals. With its global organization, Lurgi has serviced its customers around the world for more than 100 years.

Oil-Dri Corporation of America, 410 North Michigan Ave. Ste. 400, Chicago, IL 60611 USA. Oil-Dri Corporation of America is featuring a full spectrum of specialty adsorbents including Pure-Flo™, Pure-Flo Supreme™, and Pure-Flo Perform™ bleaching adsorbents for the purification of fats, oils, and oleochemicals, and Select™ products for the removal of soaps, metals and phospholipids.

Plant Maintenance Service Corp., 3000 Fite Road, P.O. Box 280883, Memphis, TN 38168-0883 USA. Plant Maintenance Service Corporation products and services include fabrication, installation and maintenance, specializing in oil seed processing equipment, pressure vessels, heat exchangers and condensers per ASME Code, conveyors, hoppers, and bins furnished and installed, and emergency services and plant turn-arounds.

Roskamp Champion, 2975 Airline Circle, Waterloo, IA 50703 USA. Roskamp Champion is the world leader in oil seed preparation and meal processing technology. We offer a complete range of Cracking Mills, Impactors, Flaking Mills, Gyro Sifters, and Hammermills, with the endurance, reliability, and efficiencies required to compete in today's changing global economy. Roskamp Champion offers equipment and services that represent the best value package to the customer.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

March 19-21, 2000 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Tramco, Inc., 1020 E. 19th Street, Wichita, KS 67214 USA. For over 30 years, TRAMCO has been involved in the design, application, engineering, and manufacturing of the most complete line of chain conveyors, enclosed belt conveyors, and specially designed conveyor and conveyor conversions. Tramco begins with sound engineering design, producing conveyors of exceptional quality and durability.

Trinity Consultants, 12801 N. Central Expressway, Suite 1200, Dallas, TX 75243 USA. Trinity Consultants is a nationwide firm that assists industrial facilities with air quality, industrial risk management, and environmental information services. Trinity services to the oilseed processing industry include: New Source Review air permitting, Clean Air Act compliance, atmospheric dispersion modeling, NPDES Clean Water Act permitting, and Storm Water pollution prevention plans. Please visit our Web site: www.trinityconsultants.com.

Westfalia Separator, Inc., 100 Fairway Court, Northvale, NJ 07647 USA. Westfalia Separator will feature its self-cleaning miscella refining separators. Westfalia Separator, a worldwide supplier of centrifuges and high intensity mixers for the vegetable oil industry, manufactures self cleaning and solid bowl separators for a wide range of capacities in degumming, neutralization, and dewaxing.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association,
Inc.

SRRC

Southern Regional Research
Center/ARS/USDA

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

An Outlook for the U.S. Oilseeds Industry

John C. Baize
John C. Baize and Associates
Falls Church, VA

Key Points:

- **Past trends in U.S. and global oilseed demand**
- **Key factors affecting global demand today**
- **Future growth markets for oilseeds and products**



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Thriving in a Consolidating Marketplace

David Bossman
American Feed Industry Association
Arlington, VA

Key Points:

- **Food safety and consumer confidence in the supply of meat, milk, and eggs is most important.**
- **While the feed industry is changing fast there are no less tons produced, just the number of buyers.**
- **World wide markets will open and close; be ready to respond.**



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Modifying a Commodity Crop to Survive in the New Millenium—the Sunflower and Soybean Experience

Edward J. Campbell
Archer Daniels Midland Company
Decatur, IL



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

GM Crops: Current Status and Future Prospects

Dwayne R. Buxton
Agricultural Research Service, USDA
Beltsville, MD



GM Crops: Current Status and Future Prospects

Dwayne R. Buxton, John W. Radin, and Peter K. Bretting
National Program Leaders
Agricultural Research Service
U.S. Department of Agricultural

Introduction

Plant breeders have a long history of improving crops for traits such as increased yield and pest resistance through traditional plant breeding techniques. Beginning in the 1970s, a series of complementary advances in molecular biology enabled relatively easy DNA transfer between more distantly related organisms. Crop biotechnology, the application of scientific techniques to crop modification and improvement, has progressed to the point that genes now can be routinely transferred across kingdoms, e.g., from bacteria into crops. The ability to manipulate crops in an increasingly sophisticated manner through both traditional breeding and modern biotechnology may enable breeders to not only more rapidly achieve historical goals for crop improvement, but to tailor crops for specific uses that until now were unimaginable. In addition to their food, feed, fiber, and horticulture uses, crops can potentially furnish many industrial products including biofuels, biodegradable plastics, and vaccines. Although genetic modification through biotechnology is simply another research tool, its continued use in crop breeding will demand that greater attention be paid to risk assessments to meet potentially more stringent regulatory requirements, allay public concern, and safeguard the environment.

Recombinant DNA technology frequently has been referred to as genetic engineering. Organisms, such as crops, that have been genetically modified, or “transformed,” by certain modern techniques of genetic exchange are commonly termed “genetically-modified organisms” (GMO) or transgenic crop. Widespread cultivation of GM crops has caused controversy in many nations, to greater or lesser extent, depending on the ideological, sociocultural, and socioeconomic factors present. In this paper we discuss the current status and future prospects for GM crops. For additional information, please see the list of selected references at the end of the paper.

Current Status

After two decades of intensive research and development, extensive commercial cultivation of genetically engineered crop varieties commenced about four years ago. Since then, GM varieties have captured large market shares for some crops. In 1999, approximately 40 million hectares of land was planted to GM varieties of some 20 crop species, the most commercially important being cotton, corn, soybean, and rapeseed. Approximately 15% of the total area is in developing countries. The nations with significant production include Argentina, Australia, Canada, China, France, Mexico, South Africa, Spain, and the United States. The value of the global market in GM crops grew from \$75 million in 1995 to \$1.64 billion in 1998. The rate of acceptance of GM crops by U.S. farmers has been spectacular and among the fastest rate recorded for new agricultural technology. U.S. farmers have perceived that genetically modified crops provide

them improved, environmentally friendly methods for controlling pests (weeds and insects) that limit yields. The United States is by far the leading nation in GM crop production where approximately 55% of the soybeans, 25% of the corn, and 40% of the cotton grown in 1999 were GMO.

The negative views and, at times, hostility toward GM crops in Europe and other countries, and to some degree in the United States and elsewhere, threatens the continuing success of this promising technology. The reluctance of some Europeans to accept GM crops is being keenly felt by farmers in the United States, as the amount of corn exported to Europe fell significantly last year.

Potential Benefits

Genetic engineering has several advantages over traditional crop breeding methods. Genetic modifications may be more precise because in general the number of genes transferred to the recipient plant is more precise. As a result fewer unwanted genes must be eliminated during further breeding, unlike what is often the case with traditional plant breeding. Crop genetic engineering may also enable more rapid development of varieties containing new desirable traits than is feasible through conventional plant breeding. Further, the specific gene being transferred is usually known so that many of the details of the genetic change also are known, which often is not the case with traditional breeding methods where the genetic bases of the trait being manipulated maybe not be known. Finally, the ability to transfer genes at will across wide evolutionary boundaries makes available potentially the entire span of genetic capabilities among all organisms. Consequently, genetic engineering markedly expands the range of useful traits that ultimately can be transferred to new crop varieties.

To date, traits transferred across wide evolutionary boundaries (e.g., from bacteria to crops) have been most commonly insect resistance (cotton, corn, potato), disease resistance (papaya), herbicide resistance (soybean), and delayed fruit ripening (tomato). The economic benefits of these initial transgenic crops are better weed, disease, and insect control, higher productivity, and more flexible crop management. These economic benefits accrue primarily to farmers and agribusinesses, but there are also economic benefits to consumers in terms of maintaining low food prices. The broader benefits to the environment and the community through reduced use of pesticides and herbicides contribute to a more sustainable agriculture and better food security. A “second wave” of new traits being incorporated into crops by industry and public researchers will potentially convey greater direct benefits to consumers, such as food that is more nutritious with a longer shelf life.

Areas of Concern (Potential Risks)

Arguments against GM crops are diverse. They may be based on economic, political, or even religious beliefs, but the issue of safety is at the heart of the disputes. Despite 20 years of work to establish safety guidelines for the testing and release of genetically engineered crops, many strongly insist that the current approval system is flawed. Potential risks associated with GM crops have received much public attention in Europe and, during the past year, in the United States, Canada, and elsewhere. Opponents of this technology point to the risk of GM crops hybridizing with weedy relatives and creating “superweeds,” of unintended consequences such as

killing non-pest insects, like the highly publicized monarch butterfly, or even killing beneficial organisms, or that novel allergens could be inadvertently introduced into non-allergenic crops. In assessing and managing the benefits and risks associated with crop genetic engineering, several issues must be addressed to enable informed decisions about the safety of the technology.

Areas of potential risks that require additional research include the following:

- *Allergenicity*: One of the most widely cited concerns about genetic engineering is that it will introduce genes into non-allergenic foods that trigger allergic reactions.
- *Pleiotropic effects*: A second concern is that genetic engineering may have unanticipated genetic effects because untargeted gene insertions within the plant genome may cause unpredictable consequences in the plant.
- *Secondary plant metabolites*: A third concern is that the introduced genes will cause unanticipated and/or enhanced expression of secondary plant compounds with potential toxic activity (e.g., production of glycoalkaloids in potatoes or tomatoes).
- *Gene flow*: The most widely cited ecological concern about genetically engineered plants is that gene flow from GM crops to weeds could lead to substantial improvements in the weed's ability to survive, i.e., the emergence of superweeds.
- *Holistic ecological evaluations*: This concern is more than gene flow, it includes effects on non-target organisms and changes in the functioning of ecosystems.

Another potential ecological risk stems from the widespread use of GM crops with insecticidal genes is the development of pesticide resistance in insect populations exposed to the GM crops. To mitigate this risk, insect susceptible varieties are planted in close proximity to the GM varieties to reduce the opportunity of the insect population to evolve resistance.

Regulation of GMOs in the USA

In the United States, GM crops are extensively tested before being marketed. The National Academy of Science played a leading role in developing this process through its study and publication of *Field testing of genetically modified organisms: Framework for decisions*, published in 1989. At present regulation of GMOs in the United States has focused on the end product rather than the production process itself. This has resulted in a regulatory framework that builds on existing conventions rather than establishing new ones, and, within that framework, reducing regulation of products once they have been determined to be of low risk. The concept of *substantial equivalence* between new and traditional products has been the basis for determining what safety tests are needed before commercialization of products from GM crops. The U.S. regulatory system for review of field testing and release of GM crop varieties has been in place since 1986. More than 22,000 field tests have occurred and some 50 products have been reviewed and moved into the marketplace. The U.S. regulatory process involves several Federal agencies. USDA-APHIS oversees and regulates certain environmental issues such as transgene escape and spread through pollen or other vectors. FDA regulates human health issues such as potentially adverse effects of altered properties of food. EPA regulates the ecological consequences of genes that encode pesticides. APHIS has deregulated 13 transgenic crops including the oilseed crops flax, rapeseed, and soybeans. With the growing concern over the adequacy of the regulation of GMOs, the National Academy of Science announced in December 1999 that it will review the scientific process for decision making by APHIS with

emphasis on non-target organisms.

Precautionary Principle

A recent development, partly in response to the negative public reaction to the increasing cultivation of GM crops, has involved several nations, especially in Europe and Japan, proposing to label biotechnology-based products, with the objective of enabling consumers to choose whether to consume food that contains ingredients derived from GM crops. Some regulatory authorities have incorporated the *precautionary principle* into regulatory requirements of GMOs. As framed at the 1992 United Nations Earth Summit in Rio de Janeiro, the precautionary principle holds that where there are threats of serious damage, lack of scientific certainty will not be used as reason for postponing cost-effective measures to prevent environmental degradation. This approach is based on the idea that not enough may be known about long-term adverse effects of GMOs, and thus use of GMOs requires extensive prior evidence of the safety of biotechnology-based products for human health and the environment. The thought is that if an error is to be made, it should be on the side of keeping GMO crops from commercialization.

Why the Backlash

In the developed world, food is relatively plentiful. Since Thomas Malthus first proposed his doomsday scenario 200 years ago, agricultural production in the developed world has almost continuously increased faster than demand despite population growth, resulting in today's imbalance between supply and demand. The consequence of burgeoning commodity production is low prices. At the same time that crop productivity is expanding, the channels for supply of inputs to farmers (seeds, fertilizers, etc.), as well as the channels for sale and distribution of their products, are rapidly being consolidated into multinational companies. The number of seed companies has greatly contracted during the 1990's, so that six multinational companies now are dominant across the globe. The rights to much of modern biotechnology is owned and controlled by these large companies. The growing dependence upon proprietary biotechnology for seed production has created some unease that the biotechnology itself contributes to the growing domination of large multinational companies.

With this backdrop, it is not surprising that consumers in developed nations may be wary of biotechnology. In particular, the first generation of genetically-engineered crops is not different in terms of food quality traits that make them more valuable to consumers. Rather, they produce a commodity just like the conventional product, which is viewed by some as unneeded in a world of surpluses. Indeed, in some parts of the world, where food safety crises are more commonplace than in the United States, the proffered justification for agricultural biotechnology is considered weak. Instead, consumers tend to mistrust the large multinational companies.

Throughout Europe and other areas that are reluctant to accept agricultural biotechnology, there is nonetheless enthusiastic acceptance of biotechnology incorporated into medicine. The difference is that medical biotechnology has developed products and therapies that are not available through any other means. Agricultural biotechnology may be widely embraced at some future date, when it yields new products that offer immediate consumer benefits that cannot be achieved by conventional means. The first of these are already being discussed in the literature. e.g., rice that is greatly enriched in vitamin A. When these second-generation products are

available and the direct benefits to consumers are readily apparent, it is likely that their acceptance will increase.

First rising in the United Kingdom with headlines warning of the horrors of foods from GM crops, the public fervor spread through Europe, leading the European Union to suspend the introduction of new GM crops pending new legislation, which could be three years away. The resistance even reverberated in the United States, where the GMO revolution had been proceeding fairly silently. In 1999, U.S. farmers, who planted much of their corn, cotton, and soybean fields with transgenic crops, watched with dismay as their export markets shrank. At recent public hearings organized by the FDA, many speakers voiced concerns that GM crops might be hazardous to human health or could cross-pollinate with wild plants and create superweeds. This eruption of public feeling also was fueled by critical studies published in 1999. One showed, for example, that monarch butterfly caterpillars in the laboratory died when fed transgenic pollen containing Bt (*Bacillus thuringiensis*). Another reported that rats' gut linings were somewhat swollen after the animals ate transgenic potatoes. This work was preliminary and controversial. Subsequent studies have generally shown that consumption of Bt pollen by the caterpillars would be minimal in natural settings, and Britain's Royal Society called the potato study "deeply flawed."

Future Prospects

Some positive actions may come out of the current debates over GMOs. These could be even better ecologically-sound ways to guide the technology to avoid problems like those raised in the debates. USDA Secretary Dan Glickman recently announced the appointment of a 25-member Advisory Committee on Agricultural Biotechnology to help guide USDA in this area. The committee includes both supporters and critics of this technology.

Labeling

The current policy in the United States on labeling of GM products was established after extensive hearings in 1992 by FDA. This policy states that foods developed through genetic engineering will be labeled only if they have been changed from "non-genetically engineered food" in some material way. For example, labels will be needed if the nutritional content were significantly altered or if allergens have been introduced. This labeling policy is now under review by FDA and Congress because of the extensive debate on the benefits and risks of GMOs. The debate includes the issue of whether product labeling should be mandatory or voluntary, what information should be on the label, and whether labeling is feasible for bulk commodities that may contain a mixture of GMO and non-GMO crops. The Codex Alimentarius Commission and OECD are currently studying standards for labeling.

Some of the current discussion is based on consumer preferences, rather than faulty food safety or environmental safety regulations. For example, some consumers prefer to avoid foods produced through genetic engineering for ethical, environmental, or social reasons. Because the current FDA policy is based on the end product, rather than the process, current guidelines for food labeling do not satisfy the needs of these groups of consumers. Voluntary labeling by

industry will likely happen in the U.S. The result may be at least two product streams, one with categories as “GM” and the other as “GMO free.” The GMO-free stream will likely incur extra costs associated with segregation and testing. Details that remain unresolved include the permissible level of contamination, how to handle processed plant materials, and how to adequately conduct testing.

Many issues and questions must be resolved before agreement can be reached on labeling. Some are discussed below.

Definition of what is being labeled. The understanding of what constitutes “genetic engineering” or what is a “GMO” varies greatly. Some definitions of biotechnology are very broad, typically including the use of all laboratory-based technology to modify plants. There must be generally accepted, common definitions of what aspects of genetic engineering or biotechnology trigger the need for labeling. For many groups calling for mandatory labeling, this criterion seems to be the introduction of any genetic material from other species through genetic engineering.

Methods for determining GMO content. Labels must be based on clear and consistent guidelines for determining GM content. What about processed food? If only the oil from GM plants exists, containing no protein or DNA from the introduced gene(s), is labeling required? New methods for altering genes, e.g., “genetic surgery” (chimeroplasty), present the prospect of foods that have been genetically modified in the laboratory without introducing foreign DNA. Will these require labeling?

Threshold concentrations for labeling. A zero-tolerance standard for “non-GM food” is likely unachievable for many commodities; therefore standards must be adopted that are feasible and that meet the public’s need. Also, should meat or milk products be labeled when the animals have been fed GM crops. Verification will be more complex because it is unlikely that there will be any diagnostic differences in the meat or milk from the animals that have been fed GM crops.

“Positive” versus “negative” labeling. With positive labeling, the label would state that the product “might” contain GMOs. This type of label may not help consumers make informed choices. In other cases, especially where biotechnology has heightened consumer appeal of the product (such as if the GM product contains improved nutrition), the label could state that it “does” contain ingredients derived from modern biotechnology. Positive labeling may become more commonplace as food producers start using genetically-engineered ingredients that provide direct consumer benefits.

The second, alternative approach is to develop a system of negative labeling. In this case, the label on the product would indicate that the food “does not” contain any ingredients derived from genetically-engineered crops. This would be analogous to an organic food label that states that the crops were grown without the use of pesticides and other categories of chemicals.

Voluntary versus mandatory labeling. Probably the best way to satisfy those who want labeling is to provide a system of voluntary negative labeling for foods *not* produced through biotechnology. If the demand is strong enough, the market will grow as it has done for organic

foods.

Future Potential of Biotechnology

Genetically-engineered crops have the potential to revolutionize the ability of agriculture to better meet the caloric, nutritional, and other needs of humankind throughout the world. This technology takes advantage of the rapid progress being made in mapping genes and identifying their functions in plants and animals, a field of science termed "genomics." Further advances in biotechnology may yield crops with wider ranges of desired traits, some of which are of more direct interest to consumers, such as those that will confer improved nutritional quality. For example, numerous genes have been identified that modify and enhance the composition of oils, proteins, carbohydrates, and starch in food/feed grains and in root crops. Genes encoding beta carotene/vitamin A formation has been transferred from daffodils and bacteria into rice. This trait could enhance the diets of the 180 million children who suffer from the vitamin A deficiency that leads to two million deaths annually. Similarly, introducing genes that increase available iron levels in rice threefold is a potential remedy for iron deficiency that affects more than two billion people and cause anemia in about half that number. Genes have been introduced into plants to produce vaccines and biodegradable plastic.

Applications of biotechnology and genetic engineering in agriculture are in their infancy. Most current GM plant varieties are modified only for a single trait. Soon we may have the ability to readily incorporate multiple genes at a time as has been the case with beta carotene encoding genes in rice. The rapid progress being made in genomics will enhance plant breeding as the functions of more genes are identified. This may enable more successful breeding for complex traits such as drought and salt tolerance, which are controlled by many genes. With improvements through biotechnology, crops have the potential to supply many industrial products such as lubricants, plastics, surface coatings, resins, inks, polymers, and fibers. Recognizing the importance of this need, President Clinton signed the Executive Order on Biobased Products and Bioenergy on August 12, 1999. This Executive Order sets a goal of tripling U.S. use of biobased products and bioenergy by 2010. Meeting this goal will require a substantial commitment to agricultural biotechnology.

Selected References

Dunwell, Jim M. 1999. Transgenic crops: The next generation, or an example of 2020 vision. *Annals Bot.* 84:269-277.

Ferber, Dan. 1999. GM crops in the cross hairs. *Science* 286:1662-1666.

Macilwain, Dolin. 1999. US food-safety body hears protests over genetically modified food. *Nature* 402:571.

Millstone, Erik, Eric Brunner, and Sue Mayer. 1999. Beyond 'substantial equivalence.' *Nature* 401:525-526.

Various Authors. 1999. The plant revolution. *Science* 285:367-389.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Washington Perspective—Regulatory, Environmental, trans Labeling

Robert Reeves
Institute of Shortening and Edible Oils, Inc.
Washington, DC

Key Points:

- Environmental regulations continue to expand in scope, and regulatory allowances of pollution emissions into the air, water or land continue to be restricted. The status of these rules will be discussed.
- The FDA recently announced its proposed regulation requiring the labeling of foods containing *trans* fatty acids. The basic requirements of these proposed rules and their effects on the fat and oil industry will be outlined.
- OSHA recently announced its long awaited "ergonomics" proposed regulation. Its far-reaching provisions have the potential to affect many aspects of the fat and oil industry.



Update on Edible Oils Regulatory Issues

By

Robert M. Reeves

Institute of Shortening and Edible Oils

AOCS / NCPA Oilseed Conference

New Orleans, Louisiana

March 20, 2000

Biotechnology

I. U.N. Biosafety Protocol Agreement

A. General Information

1. Held in Montreal, January 24-28, 2000.
2. Attended by 138 countries, tentative agreement reached.
3. Must be ratified by at least 50 countries to become effective. (Ratification is a 2-3 year process.)
4. Designed to preserve biological diversity.

B. Preamble

1. Legitimizes world trade in genetically modified products by recognition of risks and benefits.
2. Emphasizes that protocol does not override other international pacts and obligations with organizations such as the World Trade Organization.

C. Major Provisions

1. Establishes biosafety clearing house for receipt and transfer of information.
2. GM materials intended for introduction to environment required to have advance informed agreement (AIA) prior to shipment. GM materials intended for food, animal feed, and processing not subject to AIA (oilseed meal and oil).
3. GM materials intended for food, animal feed, and processing must be accompanied by documentation stating that the shipment may contain GMO's.
4. "Precautionary Principle" integrated into text of agreement therefore approvals by importing countries may not be based solely on scientific principles.
5. Importing countries may require approval of new biotech varieties under their laws and regulations but approvals not required by protocol.

II. Organization for Economic Cooperation and Development (OECD) Conference

A. General Information

1. Held February 28 – March 1, 2000, Edinburgh, Scotland.
2. Arranged by "Group of Eight" to examine ethical, environmental, social and political impacts of food biotechnology.
3. Attended by 400 scientists, academicians, regulators and environmentalists.

B. Results

1. General consensus that no medical evidence to date indicates that GM foods are not safe to eat, but more long term testing is needed.
2. Consumers have doubts about the safety of GM food; therefore, science needs to be shared in a more transparent way.

C. Implications

- Precautionary Principle likely to be strongly advocated for inclusion in Codex standards (e.g., Codex Committee on General Principles).

Trans Fatty Acid Labeling

I. FDA Proposal on Trans Fat Labeling (11-17-99)

A. Background

- Proposed regulation based on premise that scientific studies show trans fats raise LDL cholesterol similarly to saturated fats, therefore, they increase risk of coronary heart disease.

B. Basic Content

1. Inclusion of trans fats in the nutrition labeling of foods.
2. Placement of limits on trans fat content in foods making nutrient content or health claims.

C. Major Provisions

1. Trans fat included in saturated fat category
 - When trans fats are present, an asterisk is placed after the term “saturated fat” which refers to a footnote giving the amount of trans fat in grams per serving (see attachment 1).
2. “Low saturated fat” claims
 - Less than 0.5 g. trans fat and 1 g. or less of saturated fat.
3. “Reduced saturated fat” claims
 - At least 25% less saturated and trans fat combined in addition to a 25% reduction of saturated fat.
4. Cholesterol claims
 - 2 g. or less of saturated and trans fat combined.

5. "Trans free" claim

- Less than 0.5 g. of trans fat and less than 0.5 g. saturated fat per serving.

D. Comment period ends April 17, 2000.

Occupational Safety and Health

I. Ergonomics Regulation proposed by OSHA, U.S. Dept. of Labor (11-23-99)

A. Background

1. Major goal of organized labor.
2. In development for over nine years.
3. Designed to address repetitive motion and odd angle lifting injuries.

B. Major Provisions

1. Management leadership and employee participation.
2. Hazard information and reporting.
3. Job analysis and control.
4. Training.
5. Musculo-skeletal disorder management.
6. Program evaluation.

C. Industry Concerns

1. Lack of scientific evidence supporting need for regulation.
2. Potential conflict with other laws (e.g., Workers Compensation Act).
3. Cost of program.
4. Vagueness regulatory language open for misinterpretation.

II. OSHA withdraws workplace safety guidelines for telecommuters.

A. Letter of guidance placed on Internet by OSHA in November 1999.

B. Newspaper article brings issue public in January 2000.

C. Guidance letter essentially stated OSHA has regulatory oversight of workers conducting business at home.

D. Letter removed by OSHA within 48 hours of public announcement after firestorm of protest by industry.

Environmental Rules

- I. High Production Volume (HPV) Chemicals (1 million lbs./yr.)
 - EPA seeking safety testing of over 2,800 chemicals it claims have not been adequately tested.
- II. Inventory Update Regulation (IUR)
 - EPA proposing to expand requirements of industry to report the presence of certain chemicals at industrial facilities including details of amounts, characterization, concentrations, commercial uses and worker exposure.
- III. MACT Standard for n-Hexane Emissions
 - A. EPA finalizing regulation establishing limits on hazardous air pollutants including n-hexane. (See tentative limits in attachment 2.)
 - B. Edible oil crushing industry has worked with EPA over past five years negotiating regulation.
 - C. Final rule anticipated November 15, 2000.
- IV. Industrial Combustion Coordinated Rulemaking (ICCR)
 - EPA establishing standards for industrial combustion air pollutants from sources such as combustion turbines, boilers, combustion engines, process heaters and incinerators.
- V. Appeals Court Overturns PM_{2.5} and Ozone Proposal
 - A. EPA proposed revision of National Ambient Air Quality Standards for Particulate Matter (PM) and Ozone on June 18, 1997.
 - PM, from 10 microns diameter size to 2.5 microns.
 - Ozone, from 0.12 ppm over 1 hour to 0.08 ppm.
 - B. U.S. Court of Appeals in May 1999 opined that EPA's proposal was unconstitutional in that it did not use a set of decision principles in creating the standard and did not consider any beneficial effects of either PM or ozone.

Codex Alimentarius Commission

- I. Codex Committee on Fat and Oils (CCFO)
 - A. Draft Code of Practice for the Storage and Transport of Fats and Oils in Bulk finalized through step 8, November 1998.
 - Code adopted by Codex Commission June 1999.
 - Included language allowing use of thermal heating fluids in edible oil facilities if agreed upon by contracting parties and safety assured by inspection and risk evaluation.
 - B. Compositional standards for fat and oil products.
 - C. Fatty acid standards for source oils.
- II. Codex Committee on Food Hygiene (CCFH)
 - Draft Code of Hygienic Practice for the Transport of Bulk and Semi-Packed Food (at step 8)
 - A. Contains language identical to that in the CCFO relative to the use of thermal heating fluids.
 - B. Allows non-dedicated transport of liquid, powdered and granular foodstuffs provided “principles such as HACCP” demonstrate that dedication is unnecessary.
- III. Codex Committee on Food Labeling (CCFL)
 - 1. General Standard for the Labeling of Prepackaged Foods contains “Draft Recommendations for the Labeling of Foods that can cause Hypersensitivity.” (Adopted in Ottawa, April 1999.)
 - List of allergenic foods includes “peanuts, soybeans and products of these.” (May be interpreted to include refined peanut and soybean oils.)
 - Clinical study being performed in U.S. to provide additional scientific information.
 - 2. Standards for the labeling of foods derived from biotechnology.
- IV. Codex Committee on Food Additives and Contaminants (CCFAC)
 - Considering a revised standard for lead content in edible oils and fats (from 0.1 ppm to 0.05 ppm) (at step 6).

V. Codex Committee on General Principles (CCGP)

- “Precautionary Principle” likely to be proposed for inclusion pursuant to content of biosafety protocol.

International Maritime Organization (IMO)

Revision of MARPOL Annex II

(The International Convention for the Prevention of Pollution from Ships)

I. Recategorization of Noxious Liquid Substances (NLS)

- A. Establishment of new pollution categories for NLS using hazard profiles based on:
- B.
 - 1. Chemical and physical properties.
 - 2. Toxicity
 - 3. Marine pollution factors.
- C. Three pollution categories being considered instead of five (current).

II. IMO Industry Working Group

- A. Met in London, October 14, 1999.
- B. Attended by APAG, FEDIOL, FOSFA, IFMA/IMACE, IFOMA, NIOP, NRA and PORIM.
- C. Collecting data required to adequately categorize fats and oils.
- D. Determined data on toxicity and environmental effects is lacking.

III. Target Date for Completion – 2002

- Limited funding by IMO may delay completion.
- Industry working group striving to complete collection of hazard profile data to ensure appropriate categorization of fats and oils.
- Industry participation vital to completion of process.

Nutrition Facts

Serving Size 1 Tbsp (14g)

Servings Per Container 32

Amount Per Serving

Calories 100 **Calories from Fat** 100

% Daily Value*

Total Fat 11g **17%**

Saturated Fat** 4g **20%**

Polyunsaturated Fat 3.5g

Monounsaturated Fat 3.5g

Cholesterol 0mg **0%**

Sodium 115mg **5%**

Total Carbohydrate 0g **0%**

Protein 0g

Vitamin A 6%

Not a significant source of dietary fiber, sugars, vitamin C, calcium and iron.

* Percent Daily Values are based on a 2,000 calorie diet.

**Includes 2g trans fat.

Attachment 2

Seed Type	Tentative Allowable Solvent Loss (gallons/ton)	
	<u>Existing</u>	<u>New</u>
Corn germ, wet-milling	0.4	0.3
Corn germ, dry-milling	0.7	0.7
Cottonseed >120,000 tons	0.5	0.4
Cottonseed < 120,000 tons	0.7	0.4
Flax	0.6	0.6
Peanut	1.2	0.7
Canola	0.7	0.3
Safflower	0.7	0.7
Sunflower	0.4	0.3
Soybean, conventional*	0.2	0.2
Soybean, specialty	1.7	1.3

* Combination of Soybean Conventional and Specialty is ratio or minimum limit 0.25.

Minor oilseeds to be determined by states

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

ARS Research Programs in Oilseeds Quality and New Uses

Frank Flora
Agricultural Research Service, USDA
Beltsville, MD

Key Points:

- **ARS has a significant commitment in oilseeds research.**
- **ARS is changing the way it does business.**
- **ARS is seeking stakeholder input to ensure program relevance.**



ARS Research Programs in Oilseeds Quality and New Uses

Frank Flora
National Program Staff
Agricultural Research Service, USDA
Beltsville, MD 20705-5139

49th Oilseed Conference
March 19-21, 2000
New Orleans, LA

In 1999, the Agricultural Research Service (ARS), the principal research agency for the U.S. Department of Agriculture, invested more than \$39 million and the equivalent of 138 full-time scientists on research related to oilseeds and oil crops.

I will focus this discussion on research conducted by ARS in the areas of quality measurement and preservation of oilseeds and oilseeds products, and oilseeds processing and utilization, including for biofuels. In so doing, I will describe how ARS has reorganized its research portfolio under 23 national programs and how the Agency has interacted with customers and stakeholders and solicited their input on these programs.

Prior to 1998, the ARS research program was divided into six objectives for research planning and resource allocation purposes:

- 1) Soil, water, and air
- 2) Plant productivity
- 3) Animal productivity
- 4) Commodity conversion and delivery
- 5) Human nutrition
- 6) Integration of systems.

The objectives were further divided into scientific approaches, then into approach elements. Crosscuts were established to relate the ARS program to the Department of Agriculture strategic plan and the Research, Education and Economics Mission Area plan.

A couple of years ago, ARS began reorganizing its research under what are now 23 national programs designed to provide a better vehicle through which to plan, allocate resources, demonstrate accountability, and communicate ARS research priorities and accomplishments to customers and stakeholders and seek their input on these programs. Descriptions of these 23 "National Programs" can be found on the ARS homepage at <http://www.ars.usda.gov/>. The national programs are managed by teams of ARS national programs leaders, many of whom are members of several national programs. Many ARS research projects have components in more than one national program. Thus, the programs are interrelated.

Oilseeds research at ARS is conducted under several national programs. I will focus the rest of the discussion on oilseeds research conducted under ARS National Program 306, Quality, Marketability and New Uses of Plant and Animal Products and National Program 307, Bioenergy and Energy Alternatives.

There are 36 research projects in these two national programs that address quality and utility issues for oilseeds and oil crops. These projects represent an investment of \$12.4 million and the equivalent of approximately 51 full-time scientists. These include projects aimed at:

- improving the process efficiency and the utilization of cottonseed products,
- finding economical natural product substitutes for synthetic pesticides to prevent fungal infection and mycotoxin contamination of cottonseed,
- improving peanut market quality, value and handling efficiencies,
- designing efficient mycotoxin sampling plans for peanuts to reduce misclassification,
- understanding effects of processing on peanut allergenicity,
- developing new or improved value-added food and industrial products, chemical intermediates and biodiesel from vegetable oils and proteins through biotechnological, biocatalytic and chemical means, as well as through innovative processing,
- improving quality and stability of edible oils,
- developing new industrial products from alternative and new crops, and
- providing composition analysis to public soybean breeders in the U.S.

For the last year, ARS has been sponsoring workshops to implement the national programs. Customers, cooperators and stakeholders were invited to these workshops and asked to comment on the organization, scope and proposed direction of the program and to identify problems and researchable issues that might be addressed under the program. A workshop was held in April of 1999 with stakeholders of ARS National Program 307 which deals with bioenergy and energy alternatives, including biofuels. Three workshops were held to interact with the stakeholders of National Program 306 which deals with issues of quality, processing and new uses. One of these workshops was held in November 1999 in St. Louis, MO, and was focused on issues related to quality and utilization of cereals, oilseeds, and new crops. Stakeholders at the St. Louis workshop were asked to consider the following questions in two breakout sessions:

What pressing problems will American agriculture face in the future?

What roles does research related to quality enhancement/maintenance and utilization play in solving them?

Where does ARS fit in this picture?

and

Is National Program 306 properly focused on the correct questions?

What research priorities should the program address?

Does it provide an appropriate platform for partnering with ARS or non-ARS cooperators?

Some of the issues the stakeholders advised ARS to consider, and which transcend the bounds of National Programs 306 and 307, included:

- ARS should maintain a strong base of longer-term, fundamental research, balanced with applied research programs.
- The ARS research focus should be regional, national and/or global.
- ARS research should be coordinated with universities, industry and other federal agencies.
- ARS research and information dissemination should be holistic and seamless across the National Programs.

- ARS research should consider economic feasibilities and market opportunities.
- ARS research should reflect awareness of and sensitivity to consumer issues.
- ARS research on crop improvement should include traditional breeding as well as genetic modification.
- ARS should enhance its outreach efforts to promote support for its programs and to educate the private sector on the value and opportunities for partnerships with ARS.
- ARS should seek continued involvement of stakeholders to assure program relevance.
- ARS should generate baseline data for setting environmental regulations.

Research needs identified by stakeholders and relevant to National Programs 306 and 307, relating to issues of quality and utility, included:

- Improved understanding of structure/function relationships
- Quality attribute identification, detection, quantification, and tracking from field to fork
- Rapid and nondestructive tests (GMO's varieties)
- Phenotypic markers for high value traits
- Methods to detoxify mycotoxins
- New value-added biomaterials and co-products
- Reduced costs for biobased products
- Products and processes with clear human health benefits
- Safer, more environmentally friendly processing technologies and products
- Domestic biobased replacements for imports, particularly, petroleum
- Industrial model of farming where crops are viewed as delivery systems for specific traits
- Improved cost efficiency, cold flow properties, emissions, and stability of biodiesel

ARS is developing five-year research plans for each of the national programs using stakeholder input from the national program workshops. These national program research plans will in turn be used in a newly established ARS peer review process of research projects based on the national programs.

The workshops are not the only source of stakeholder input and involvement in ARS research programs, however. ARS meets with stakeholders in informal and formal settings, some on a regularly scheduled basis, others on a need or demand basis. In 1998, ARS solicited input from stakeholders on the proposed program statements for the national programs. ARS has partnered with several commodity organizations to identify research needs. The ARS strategic plan for peanut research, for example, is based on interaction with the Peanut Foundation and is integrated with the Peanut Foundation strategic plan. Interested parties can comment in writing or submit comments on the national programs through the Internet. ARS plans to continue seeking input from stakeholders for its national programs to ensure that its research programs are properly focused and relevant to current and future needs of producers, processors, marketers, and consumers.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Specialty Oils—Characteristics and Concerns

Kathleen Warner
Food Quality and Safety Research
NCAUR/USDA
Peoria, IL

Key Points:

- **Fried food flavor in fried foods decreases with increasing oleic acid in oil.**
- **Oleic acid produces volatile compounds with little or no fried food odor.**
- **Oil stability could be increased if tocopherol levels and ratios were optimized.**



Specialty Oils-Characteristics and Concerns

Kathleen Warner

Food Quality and Safety Research, U.S. Department of Agriculture
National Center for Agricultural Utilization Research, Peoria, IL 61604

Introduction

Oils from oilseeds with modified fatty acid compositions have tremendous potential for food applications now and in the future because they can be either more healthful and/or have greater stability than hydrogenated oils or polyunsaturated oils currently in use. The variety of specialty oils has grown rapidly over the past 10 years and is still expanding to include oils such as low linolenic acid canola and soybean oils; high oleic sunflower, safflower, soybean, and canola oils; mid-oleic corn and sunflower oils and high stearic and high palmitic soybean oils. Food scientists, oil chemists, and plant geneticists have collaborated in the past to determine the end-use performance of these oils; however, future collaborations should be directed toward selecting optimum oil compositions for future oilseed cultivars.

Improving the oxidative stability of oils has been one of the goals of oilseed fatty acid modification. Based on early research (1) that showed that linolenic and linoleic acids contributed to lower oil stability, geneticists have tried to develop cultivars with as little linoleic and linolenic acid as possible. In addition, saturated fatty acids, palmitic and stearic were targeted for reduction for health reasons. The end result of such modifications of these fatty acids is an oil composed primarily of oleic acid. Initially these fatty acid changes were viewed as not only providing a stable salad oil, but also an oil with maximum stability during frying. As a result, plant breeders have developed oilseed cultivars with very high levels of oleic acid of 85-90% and low levels of palmitic, stearic, linoleic and linolenic acids. Although this fatty acid composition may contribute significantly to frying oil stability, the high levels of oleic acid with corresponding low levels of linoleic acid can limit the intensity of desirable fried food flavor in fried foods (2-3).

Deep fat frying is used in restaurants and in the snack food industry to prepare fried foods and accounts for much of the market for cooking and frying oils (Fig. 1). Frying is a popular food preparation because it imparts desirable fried food flavors to food products not produced by other cooking procedures. Hydrolysis, polymerization and thermal oxidation are chemical and physical processes that occur during frying; all of which can affect the flavor of fried food both positively and negatively. We have studied the chemical basis for the positive flavor developments in fried foods. Cottonseed oil with its high (52%) level of linoleic acid is considered to be the industry standard for producing food with desirable fried food flavor. Previous research (2) showed that as the amount of oleic acid is increased and the amount of linoleic acid is decreased in an oil blend, the quality and fried food flavor intensity of potato chips decreased (Fig. 2). This may be explained in part from research (4) on the odor analysis of volatile compounds that breakdown from trilinolein heated only a short time to a total polar compound level of 5%. Compounds including 2,4-decadienal, 2,4-octadienal, 2,4-nonadienal, 2-heptenal and 2-octenal all produced fried food odor during gas chromatography-olfactometry.

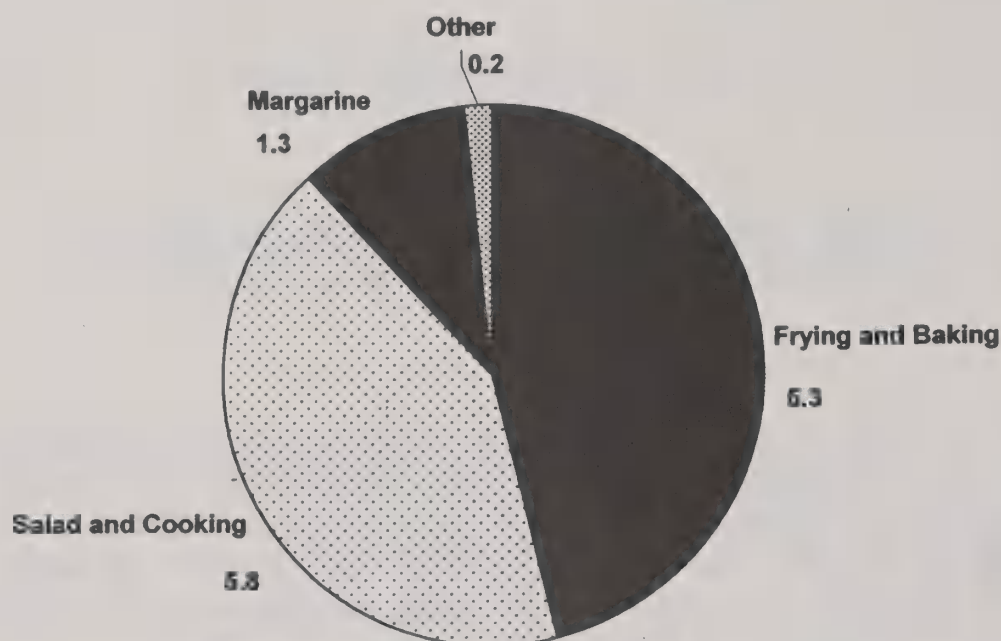


Fig. 1. Usage of Fats and Oils in the U.S. - 1997 (in billion pounds)

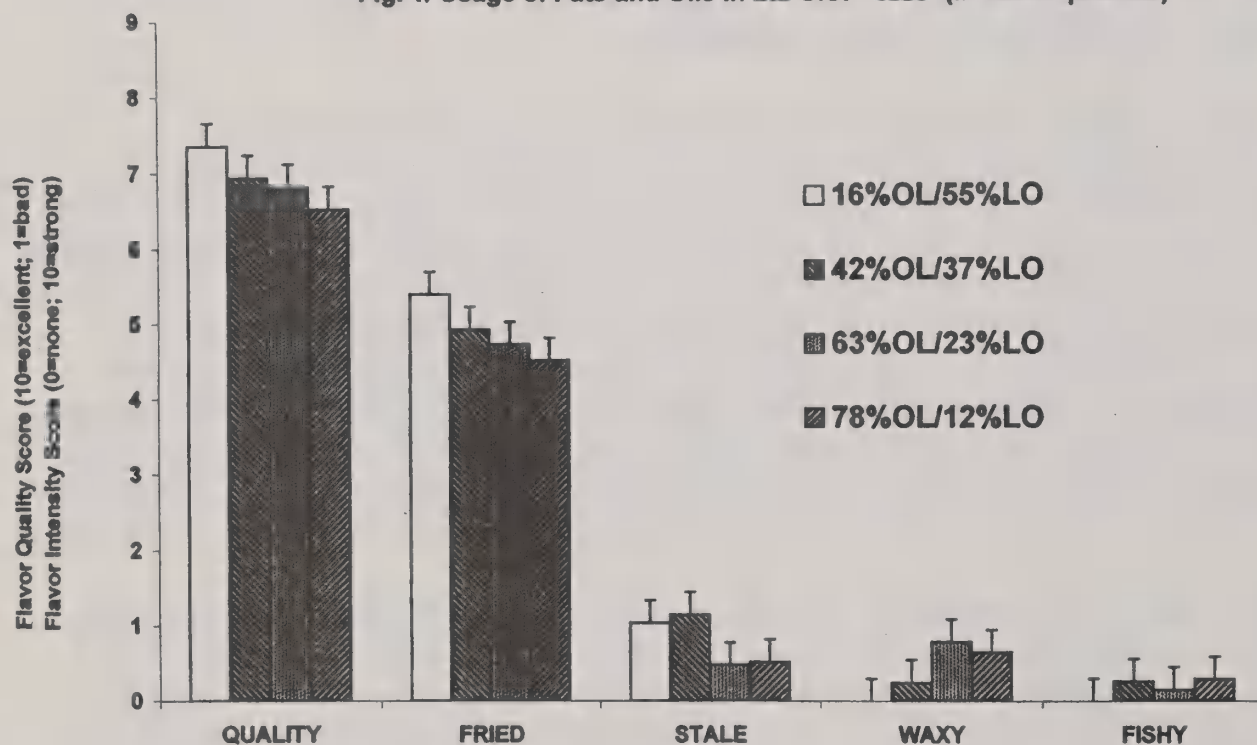


Fig. 2. Effect of Oleic and Linoleic Acid Levels on the Overall Quality Scores and Flavor Intensity Scores of Fresh Potato Chips

On the other hand, 2,4-decadienal was only produced in heated triolein after long heating to produce a total polar compound level of 30%. Studies of higher oleic vegetable oils (80% oleic) have shown that these oils had improved frying stability compared to higher linoleic acid; however, the desirable fried food odor and flavor associated with higher levels of linoleic acid was diminished in foods fried in higher oleic oils. Along with diminished fried food odor/flavor, negative odors such as fruity, plastic, acrid and waxy were characteristic of higher oleic oils during frying or heating (2-3).

Fatty acid composition of oil is well known to affect the quality and stability of oils, but fatty acid types and amounts are not the only factors that affect quality and stability. Based on our previous research (5), we know that minor oil constituents such as tocopherols play an important role in quality and stability of oils. Natural oils have a wide range of tocopherol levels (Fig. 3). For example, soybean oil is low in alpha-tocopherol and high in both gamma- and delta-tocopherols. On the other hand, sunflower is high in alpha-tocopherol and low in gamma- and delta-tocopherols. These differences in tocopherol contents may help partly explain why there are differences in the oxidative stabilities of sunflower and soybean oils (6). To study the effects of various levels and ratios of pure alpha, gamma and delta tocopherols, we added these tocopherols to purified soybean and sunflower oils and measured the flavor stability of the oils.

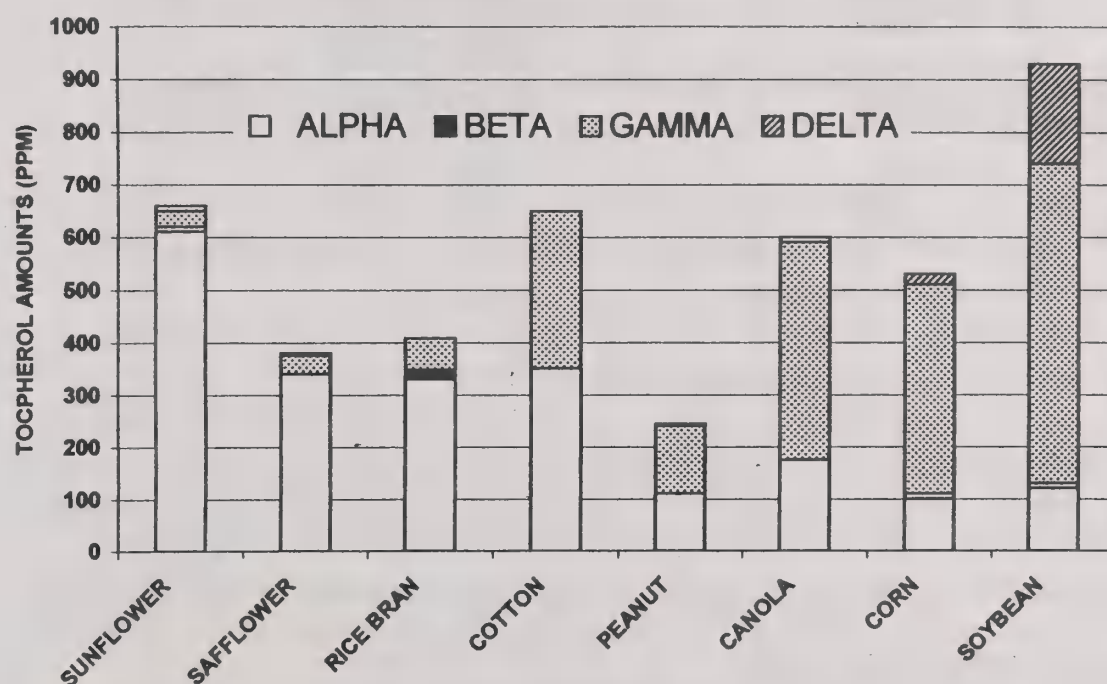


Fig.3. Tocopherol Compositions of Selected Vegetable Oils

Methods and Materials

Commercially refined, bleached and deodorized sunflower and soybean oils were stripped of naturally occurring minor constituents such as pigments, tocopherols and phytosterols through a column of 90% activated alumina and 10% carbon black. Oils were deodorized under laboratory conditions at 220°C, 3 hr with no citric acid added. Fatty acid compositions of the oils are shown in Fig 4. Pure tocopherols (alpha, gamma and delta) were added to the purified soybean oil in the ratios and levels found in soybean oil and of sunflower oil (Fig. 3). The process was repeated with the addition of pure tocopherols characteristic of soybean and of sunflower oil to purified sunflower oils. To evaluate the stability of the oils containing various levels of tocopherols, oils were stored at 60°C for 0, 2 and 4 days in dark. Oils were analyzed for flavor by a 15-member trained, experienced analytical sensory panel (AOCS method Cg 2-83) (7).

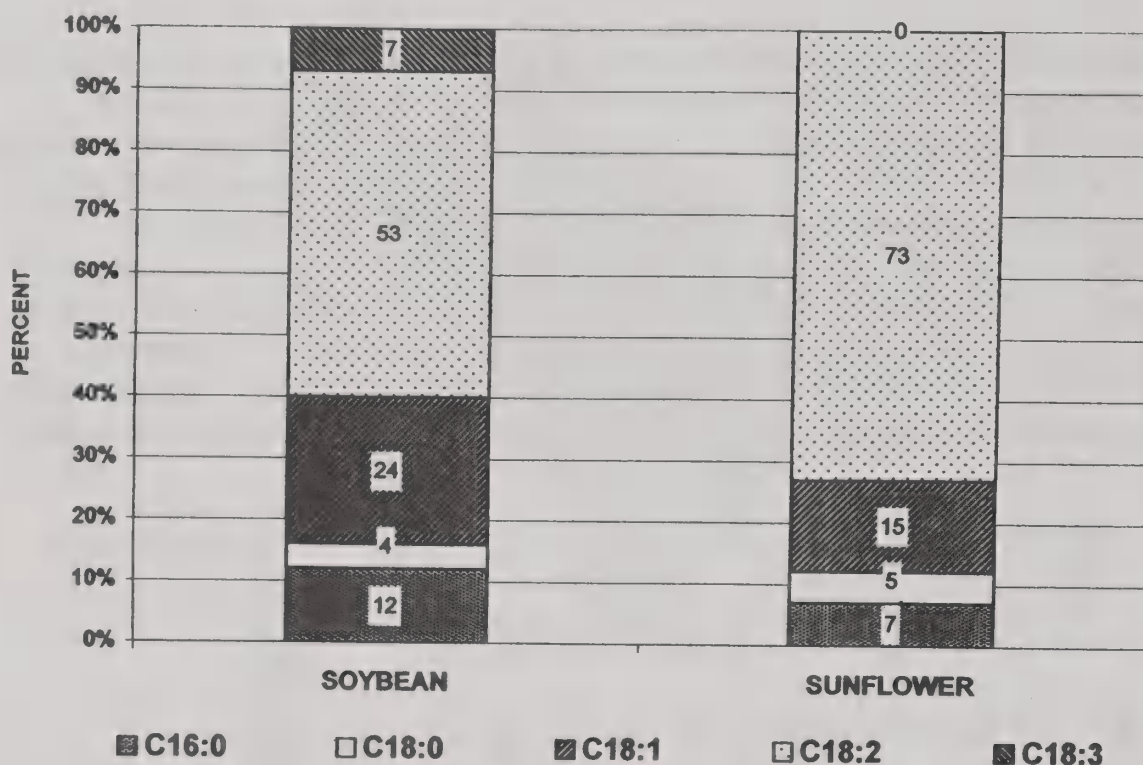


Fig. 4. Fatty Acid Compositions of Soybean and Sunflower Oils

Results and Discussion

The effects of oil type and tocopherol ratios on flavor scores are shown in Fig. 5. Both soybean and sunflower oils that contained sunflower tocopherols with their high levels of alpha tocopherol had lower flavor scores than oils with the soybean tocopherols which contained more oxidatively stable gamma and delta tocopherols. In summary, the results of these tests showed that the oxidative and flavor stability of sunflower oil could be improved if tocopherols found in soybean oil (gamma and delta) were added. On the other hand, the stability of soybean oil was decreased if sunflower tocopherols (alpha) were added.

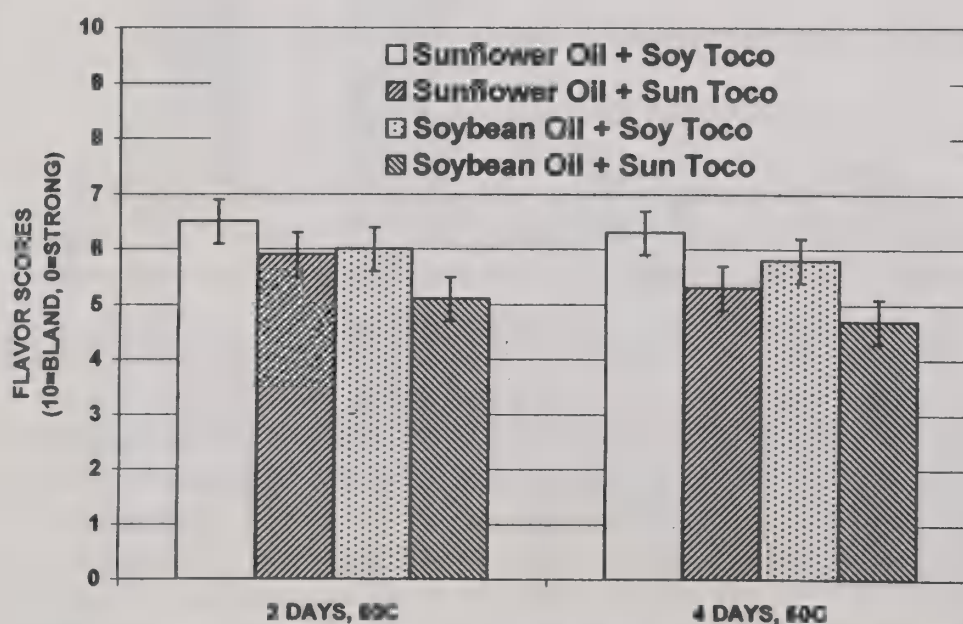


Fig. 5. Overall Flavor Intensity Scores for Purified Soybean and Sunflower Oils with Soybean or Sunflower Tocopherols

These types of additions of tocopherols to oils may not be practical on an economic basis; however, plant geneticists may consider altering the tocopherols found in oils to types and ratios of that enhance the quality and stability of oils. Now that the geneticists can fine tune the fatty acid composition of oilseeds, the next challenge should be the manipulation of minor constituents such as phytosterols and tocopherols. Tocopherol content in many oils needs to be optimized to improve oil quality and stability.

References

1. Cowan, J. C., 1966. Key Factors and Recent Advances in the Flavor Stability of Soybean Oil. J. Am. Oil Chem. Soc. 43:300A, 302A, 318A-321A.
2. Warner, K., P. Orr, and M. Glynn. Effect of Fatty Acid Composition of Oils on Flavor and Stability of Fried Foods. J. Amer. Oil Chem. Soc. 74:347-356, 1997.
3. Warner, K., P. Orr, L. Parrott, and M. Glynn. Effects of Frying Oil Composition on Potato Chip Stability. J. Amer. Oil Chem. Soc. 71:1117-1121, 1994.
4. Warner, K., W. E. Neff, and W. C. Byrdwell. Significance of Volatile Precursors and Volatile Odor Compounds in Heated Triolein and Trilinolein. Abstracts of 1999 Annual AOCS meeting, Orlando, FL, p. 25.
5. K. Warner, Measuring Tocopherol Efficacy in Fats and Oils, in Antioxidant Methodology: In vivo and In vitro Concepts, ed O. Aruoma and S. Cuppett, AOCS Press, Champaign, IL. 1996.
6. Warner, K., E. N. Frankel, and T.L. Mounts. Flavor and Oxidative Stability of Soybean, Sunflower and Low Erucic Acid Rapeseed Oils. Journal of the American Oil Chemists' Society 66:558-564, 1989.
7. Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th ed., AOCS, Champaign, IL, 1998.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Research in Alternative Solvents

Peter J. Wan

**Southern Regional Research Center
Agricultural Research Service, USDA
New Orleans, LA**

Key Points:

As an alternative solvent, isohexane

- is not a HAP
- it saves energy, and
- allows the oil mill to operate more efficiently.



Research in Alternative Solvents

Peter J. Wan
Southern Regional Research Center
Agricultural Research Service, USDA
New Orleans, Louisiana

Abstract

Research in alternative solvent systems has always been a focus for the scientists at the Southern Regional Research Center, Agricultural Research Service, USDA. Among the major solvent systems that have been investigated are: aqueous acetone, acetone-hexane-water azeotrope, ethanol, and various hydrocarbons. Each solvent system possesses its own unique set of benefits and weaknesses. So far only isohexane appears to be able to gain some approval by the oilseed industry probably because it is not a hazardous air pollutant (HAP), it could save energy and it can increase throughput of an oil mill.

Introduction

The regional research centers of the Agricultural Research Service, United States Department of Agriculture have had alternative solvent as one of their research subjects for years. The justification to sponsor research in this area may come from any one or combination of the following factors: environmental friendliness, extraction efficiency, operation safety, product quality or new products, etc. Both Northern and Eastern Regional Centers have devoted resources to supercritical CO₂ as an alternative solvent for extraction or process media for dairy protein. Each has made significant progress. Besides the high capital cost of supercritical fluid facility, making it a continuous operation appears to be the primary debacle to overcome before the commodity oilseed industry can consider its possible use. Isopropanol has been studied by the scientists at Texas A&M University during the 1990's. It was motivated by the same set of factors and was proven technically feasible but uneconomical for the oilseed extraction (1).

Alternative Solvent Research at the Southern Regional Research Center (SRRC)

Since the 1960's, scientists at the SRRC have investigated the following alternative solvents for oilseed extraction: aqueous acetone, acetone-hexane-water azeotrope, ethanol, and various hydrocarbons. The main focus with acetone was to rely on its ability to extract oil and to remove gossypol and aflatoxin from oilseeds (2-4). The idea of using acetone or acetone containing solvents as extraction solvents was shelved due primarily to problems in maintaining the three component composition and an undesirable odor of diacetyl oxides which was described as a catty odor and produced during processing (4). Although no known antinutritional problem associated with the bad odor, it made the meal unsuitable for food applications. Ethanol is biorenewable and a desirable solvent for oilseed extraction. Its technical feasibility to extract oil and antinutritional components such as gossypol and aflatoxin has been fully demonstrated (5,6). The use of ethanol, however, requires some modification of existing processing equipment. At the present time, the cost of retrofit and energy required to recover ethanol is higher than its potential benefits (5).

The environmental issues related to volatile organic compounds (VOC) and hazardous air pollutants (HAP) have once again been brought to the attention of commercial hexane users (7). Commercial hexane, a mixture of several isomers of hexane, is regulated as a VOC that

can undergo photochemical oxidation in the atmosphere to form ozone. Facilities that emit more than 100 tons per year (or less if they are in ozone non-attainable areas) are major sources. The main component of commercial hexane, n-hexane, is a neurotoxin based on animal inhalation studies and is therefore regulated as a HAP under the EPA Clean Air Act. Facilities that emit 10 tons or more of n-hexane per year are a major source. Major sources require a federal operating permit (Title V) and the solvent loss will be assessed at a fee set by the state government at over \$30-\$40/ton. Users of solvents containing n-hexane are also covered by the Toxic Release Inventory (TRI) requirement of EPA's Emergency Planning and Community Right-to-Know (EPCRA) regulations. Some oil mills are in the top 5 emitters for TRI in their states. The vegetable oil processing industry is working cooperatively in developing the maximum available control technology (MACT) standard for oilseed operations for the HAP n-hexane. This standard will require most oilseed facilities to lower their commercial hexane emissions to about 0.2 to 0.7 gal HAP/ton. For specialty soy, the maximum allowed HAP could be 1.7 gal/ton.

In an effort to search for an alternative to commercial hexane, we demonstrated that commercial isohexane, which contains less than 3% n-hexane, functioned well in laboratory and limited plant trials (8-10). The first plant trial showed a 40% savings in steam usage and a 20% throughput increase. This observation prompted a second plant trial with commercial isohexane at a 270 tons/day cottonseed oil mill. This oil mill had a new extraction - miscella refining - desolventizing/ toasting facility which was operating at slightly greater than half of its designed capacity (500 tons/day). The results obtained from the second plant trials essentially confirmed those observed from the first plant trials.

Isohexane is not covered under TRI and is not a HAP, therefore, does not require a MACT standard. The managers and operators of oil mills should also be reminded that commercial isohexane can be a cost efficient solvent under most oilseed processing conditions. The take home message from this work is that isohexane could save energy and increase the throughput rate of an existing operation without major retrofit.

References

1. Lusas, E. W. and Hernandez E. Isopropyl alcohol. pp. 199-266. In Wan, P. J. and Wakelyn, P. J. (Eds) Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils. American Oil Chemists Society Press. 1997. Champaign, IL. pp. 353. (Book Chapter).
2. Goldblatt, L. A. and Robertson, J. A. Extraction of aflatoxin from groundnut meal with acetone-hexane-water azeotrope. *Int Biodeterior Bull.* 1 (1):41-42. 1965.
3. Pons, W. A. and Eaves, P. H. Aqueous acetone extraction of cottonseed. *J. Am. Oil Chem. Soc.* 44 (7):460-464. 1967.
4. Hron, R. J., Sr. and Kuk, M. S. Acetone extracted cottonseed meals without catty odors. *J. Food Sci.* 54 (4):1088-1089. 1989.
5. Abraham, G., Decossas, K. M., Hron, R. J. and Kuk, M. S. Process engineering economic evaluation of the ethanol extraction of cottonseed: preliminary analysis. *J. Am. Oil Chem. Soc.* 68 (6):418-421. 1991.
6. Hron, R. J. Sr., Kuk, M. S., Abraham, G. and Wan, P. J. Ethanol extraction of oil, gossypol and aflatoxin from cottonseed. *J. Am. Oil Chem. Soc.* 71 (4):417-421. 1994.
7. Wan, P. J., Pakarinen, D. R., Hron, R. J. Sr., Richard, O. L. and Conkerton, E. J. Alternative hydrocarbon solvents for cottonseed extraction. *J. Am. Oil Chem. Soc.* 72 (6):653-659. 1995.
8. Wan, P. J., Hron, R. J. Sr., Dowd, M., Kuk, M. S. and Conkerton, E. J. Alternative hydrocarbon solvents for cottonseed extraction: plant trials. *J. Am. Oil Chem. Soc.* 72 (6):661-664. 1995.
9. Wan, P. J. and Wakelyn, P. J. Regulatory considerations of VOC and HAP. *INFORM.* 9(12):1155-1160. 1998.
10. Wan, P. J. and Wakelyn, P. J. Regulatory considerations of VOC and HAP from oilseed extraction plants. *Oil Mill Gazetteer.* 104(6): 15-26. 1998.

Oilseed and Oil Processing Research at ARS

P. J. Wan
Southern Regional Research Center
Agricultural Research Service, USDA
New Orleans, Louisiana

Interpretive Summary

Research in processing technology and chemistry of oilseeds and oils has always been one of the major emphases of Agricultural Research Service (ARS), USDA. For more than 50 years, scientists and engineers of the ARS have contributed to the advancement of processing technology and better understanding of the oilseed and its oils. More recent examples are the alternative solvent to commercial hexane, the processing effect on available gossypol in cottonseed meal, free fatty acid determination of cottonseed, etc. The focus of the future research will continue to be in the area of improved productivity by innovative process, product quality, analytical methods, by-product utilization and better understanding of the chemistry of oils and oilseeds.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Current Status of Membrane Technology in Oilseed Processing and Edible Oil Refining

S. Sefa Koseoglu
Food Protein Research and Development Center
Texas A&M University System
College Station, TX

Key Points:

- New membranes created new opportunities that did not exist five years ago.
- Membrane refining, degumming, and bleaching technology reduces energy usage and eliminates waste stream.
- Commercial systems may be available within a few years.



Current Status of Membrane Technology in Oilseed Processing and Edible Oil Refining

**Dr. S. S. Koseoglu
Head-Separation Sciences Program**

**Food Protein Research and Development Center
Texas A&M University System
College Station, Texas 77843-2476**

ABSTRACT

Crude oils typically are degummed, refined, bleached and deodorized to remove undesirable compounds such as free fatty acids (FFAs), phosphatide, particulates, coloring materials like chlorophylls and xanthophyll, and miscellaneous non-saponifiable materials.

Considerable amounts of energy in the form of steam or electricity are required in these processes, and each step of the edible oil process only removes one or two undesirable components. If crude oil is not properly processed, treatments during the following steps will be more difficult, and time, energy and labor consuming. In addition to the energy costs, caustic refining, water washing and bleaching steps produce various waste streams such as high BOD acidic waste water and used bleaching clay that either needs to be treated or recovered because of economical or environmental reasons.

Use of membrane separations in the edible oil industry has not materialized due to the lower stability of commercial membranes. Also, lower overall profit margins of the edible oil industry have prevented the processors from looking at more risky but promising technologies such as membranes. In recent years, the majority of membrane-based applications in the oilseed extraction and edible oil refining industry were evaluated at the laboratory-scale and only a few reached pilot plant-scale testing levels which could be commercialized within the next five years.

A variety of tests were conducted during this phase of the project to collect additional performance data on crude soybean processing by membrane technology. The specific test performed included hexane and miscella profiles, fouling tests with miscella addition, concentration profiles and lecithin purification. The effects of pressure, temperature, optimum velocity on permeate flux of miscella and selectivity were evaluated and one hundred percent of hydratable and non-hydratable phospholipids and Mg were rejected by the membrane.

The recent study indicates that membrane separation was effective for removing phospholipids and other impurities from crude cottonseed oil and has good potential for reducing steam utilization and solid waste, and completely eliminating excess water utilization.

These promising results obtained during both tests performed at cottonseed and soybean mills have shown that membrane degumming is suitable for various vegetable oils and that this technology is ready to be commercialized.

1. Introduction/Background

World consumption of vegetable oils will likely increase by more than 2% annually during the 1990s and will reach more than 80 million tons in 2005 (Morgan, and Sanford, 1989). Vegetable oil demand is expected to exceed that of protein meals.

Crude vegetable oils are produced by extraction of retreated oilseeds with hexane. The individual steps of seed preparation is directly related to seed type, moisture content, solvent ratio, type of extractor and pretreatment equipment available at that specific site. Approximately 1.51×10^9 lbs. of crude oils is processed in the US alone to prepare various types of final products such as margarine, shortenings and salad and frying oils.

The conventional crude oil refining process consists of four steps (Hoffman, 1989; Jones, and King, 1990; List, 1980; and Carr, 1978). The first step is degumming to remove the phospholipids. Crude vegetable oils can either be degummed or not degummed prior to caustic refining, depending on whether lecithin is saved and facilities are available. Next, free fatty acids (FFAs) are neutralized with a sufficient sodium hydroxide (NaOH) solution to convert FFAs into soaps. An excess of NaOH is required to reduce the color of the refined oil and ensure the removal of trace elements. Phospholipids also react with water, if not removed previously. Soaps are then removed with phospholipids by centrifugation and hot water washing. In this step, water is added at a 10-15% level at 80-90°C. Third, during the bleaching process, oxidation products are destroyed or absorbed, and pigments and trace metals are removed by adsorbents such as activated bleaching clays. The process usually takes 15-30 min at 90-95°C under vacuum. Finally, oil is steam distilled under high vacuum to strip out trace amounts of FFAs, aldehydes, ketones, and other volatile compounds, thus producing a bland flavored oil. During deodorization, the oil is heated to 230-250°C under a vacuum of 1-4 mmHg. Various unit operations are used to remove the crude oil from the seed and consequently purify the crude oil to manufacture quality products free of undesirable impurities that satisfy market demands. Although improvements have been made in process engineering and equipment design, the basic principles of edible oil refining have not changed in the last seventy years (Koseoglu, and Engelgau, 1990).

2. Membrane Technology

Simple, selective, and energy efficient membrane technology is evolving rapidly. Different components are separated according to the molecular weights or particle sizes, and the separation is somewhat dependent on the interaction of the molecules with the membrane surfaces and other components of the mixture. Microfiltration (MF) encompasses the separation of macromolecules from 200,000 to 1 million MW, ultrafiltration (UF) with molecules 30,000-300,000 MW, nanofiltration (NF) with molecules 70-15,000 MW, and reverse osmosis (RO) with ions and molecules up to 600 MW.

Applications of membrane technology in food processing are developing broadly and include processing of meat by-products, protein separations, fats and oils, milk, beverage, sugar, and fruit and vegetable juices. Membrane technology is simple, selective and energy efficient. In the dairy industry, an average membrane based plant can use a

1,000 square meter membrane area (140 membrane elements). This is directly related to each specific application and its specific site. For example: one million lbs per day whey plant utilizing membranes may cost \$700,000 and this investment may be paid back within a year. All usage given in the oils and fats industry is coming from wastewater applications. The heavy use of membranes is mainly in dairy, grain milling and beverage processing industries.

Use of membrane separations in the edible oil industry has not materialized due to the lower stability of commercial membranes. Also, lower overall profit margins of the edible oil industry have prevented the processors from looking at more risky but promising technologies such as membranes. In recent years, the majority of membrane-based applications in the oilseed extraction and edible oil refining industry were evaluated at the laboratory-scale and only a few reached pilot plant-scale testing levels which could be commercialized within the next five years (Koseoglu, S.S., et al (1997); Koseoglu, S.S., (1997); Koseoglu, S.S., (1991); Koseoglu S. S., and D.E. Engelgau, (1990) and Lin, L., et al. (1997).

Table 1 shows the current commercial and developing applications of the membranes in oilseed processing and edible oil refining. The commercial membrane separation processes are offered in the areas of nitrogen production, and waste treatment applications. Developing membrane applications in oil milling and edible oil processing are: (1) solvent recovery; (2) degumming; (3) free fatty acid removal; (4) catalyst recovery; (5) recovery of wash water from second centrifuge; (6) cooling tower water recovery; (7) protein purification; (8) tocopherol separations; (9) catalyst recovery; (10) surfactant recovery; (11) esterification; and (12) waste air treatment.

Membrane applications in edible oil processing and expected benefits are listed in Table 2. All of the unit operations listed here were evaluated individually and a larger economic impact is expected if one or two are combined.

The greatest potential for energy savings exists in replacing or supplementing conventional degumming, refining, and bleaching processes with a single step membrane separation system. A great deal of profit should be realized due to improved productivity and elimination of the chemical process. Moreover, it is emphasized that the membrane process can be carried out without water and chemicals, resulting in no wastewater streams. This would reduce costs by eliminating installations for treating effluents.

3. Recent Results:

This new membrane method has the potential to simplify the whole conventional crude oil refining process to almost a single step operation (Figure 1). The process not only removes all of the phospholipids, but also removes the majority of the coloring pigments and some of the free fatty acids. This unique process compared to conventional methods has many advantages. These are: 1) elimination of degumming process during which neutral oil loss is reduced by 80% and doesn't require phosphoric acid for non-hydratable phosphatides; 2) elimination of caustic refining that will eliminate waste waters from degumming, acidulation, water washing and drying; 3) 50% reduction in bleaching requirements and neutral oil losses; and 4) ability to utilize physical refining for high phospholipid containing oils such as soybean and cottonseed. According to the

Department of Energy study (DOE/IP 10210), the full implementation of membrane processing in the domestic edible oil refining industry will result in energy savings of 7.2 - 35.3 trillion Btu/year. In two years, 14.48 millions gallons/year of wastewater and 27,670 tons/year of solid waste will be eliminated.

a. Objectives

The research objectives of the proposed process engineering research were: 1) to identify process parameters including flow rates, temperature and pressure correlation using soybean oil miscella; 2) to collect data on fouling, membrane stability and membrane cleaning; and 3) to determine mass transfer balances.

b. Results and Discussion

A variety of tests were conducted during this phase of the project to collect additional performance data on crude soybean processing by membrane technology. The specific tests performed included hexane and miscella profiles, fouling test with miscella addition, concentration profiles and lecithin purification. Summaries of the test results are given in Tables 1&2.

Pure hexane and miscella profiles are given in Figures 1&2 show that the optimal pressure was 400 psi for this specific membrane. The permeate flux range for both hexane and miscella were 29-72 lmh and 4-40 lmh respectively. Hexane flux as expected was the highest (71 & 90 lmh) compared to all other mixtures (Tables 1&2). Tests with continuous miscella addition gave a flux of 47.1 lmh at 400 psi. During the concentration runs the permeate flux was reduced from 17.9 lmh to 9.5 lmh (Table 1). Permeate flux decreased drastically from 17.9 to 9.5 LMH as the concentration factors increased from 1 to 6. Volumetric concentration factor is the ratio of the initial feed volume to the retentate volume, and indicates the degree of separation attained.

Effects of pressure, temperature, and optimum velocity on permeate flux of miscella and selectivity was evaluated and one hundred percent of hydratable and non-hydratable phospholipids and Mg were rejected by the membrane.

In general, almost all feed components will foul the membrane to a certain extent. Fouling is partially dependent on operating parameters, particularly feed concentration, and is also time dependent. A spiral-wound membrane is reportedly one of the most compact and inexpensive membrane designs available today. In order to reduce fouling problems associated with spiral wound membranes, the crude oil feed in this study was prefiltered by microfiltration (100 μ). The flux declined rapidly in the first two hours and maintained half its initial flux for the rest of the run.

Limited cleaning studies were conducted during this study in a non-aqueous environment. The clean hexane flux on the new and the cleaned membranes were compared and showed that the original solvent flux can be restored. The results of this study indicate that membrane separation was effective for removing phospholipids and other impurities from crude soybean oil and has good potential for reducing steam utilization, and solid waste and completely eliminating excess water utilization. A

permeate flux of above 47 lmh and phospholipid rejection rate of 100% was achieved with soybean oil.

In this study, the PV value, FFA content and p-Anisidine value of oil were examined and the TOTOX value was calculated. The PV value measures the extent of primary oil oxidation products (hydroperoxides). The p-Anisidine value measures the secondary oxidation products (aldehydes), and is an indication of the harshness of processing. Severely damaged oil contains high amounts of residual aldehydes.

During membrane processing, almost no lipid oxidation took place due to the mild operating conditions. In addition, some of the oxidation products, peroxides and aldehydes, present in the crude oil were rejected by the membrane, as indicated by the lower PV value and p-Anisidine values of the permeate oil. The lecithin obtained had a higher Phosphatidyl choline content than that of conventional process. The membrane was able to concentrate lecithin to acceptable levels without membrane fouling.

c. Potential Beneficiaries:

The potential beneficiaries of the technology include edible oil refineries, oil mills, lecithin manufacturers, utility companies, food formulators and municipal wastewater treatment facilities. The details are given as follows.

Potential Beneficiaries	Benefits
Edible Oil Refineries	Reduced steam utilization, Elimination of waste water, 50% reduction of bleaching clay, Consistent quality products, Better quality products
Oil Mills	High value added products, Better price for the crude oil, Consistent quality good crude oil
Lecithin manufacturers	High quality lecithin, Natural products
Utility companies	Convert steam based plants to electricity
Food formulators	High quality oil
Municipal waste water treatment facilities	Less BOD and COD containing waste water and less volume of liquid to be treated

d. Impact On Consumers:

The impact on consumers can be summarized as follows.

- High quality products
- Natural lecithin products
- Minimal environmental impact
- Less utilization of natural resources
- Minimal dependence to petroleum based energy sources

These promising results which were obtained during the recent tests of soybean oil demonstrated that membrane degumming is suitable for various vegetable oils and that this technology is ready to be commercialized.

Table 1. Commercial and developing applications.

Commercial Applications	Membrane Technique	Developing Applications	Membrane Technique
Nitrogen Production Waste Water Treatment Miscella Filtration Oilseed Protein Recovery	GSM UF/RO MF UF/RO	Solvent Recovery Vapor Recovery Degumming Free Fatty Acid Removal Condensate Return Tocopherol Purification Catalyst Recovery Surfactant Recovery Esterification Air Purification	NF/RO NF/PV UF/NF NF RO MF/UF/NF/RO MF UF PV UF/MF

Table 2. Membrane applications and expected benefits.

Membrane Utilization	Membrane Function	Expected Benefits
Degumming	Separate phospholipids, coloring pigments and metal ions	Less neutral oil loss, ability to physical refine the oil, less bleaching clay requirements, no water wash required, produce high quality lecithin, less energy consumption
Deacidification	Remove free fatty acids from triglycerides	No chemical refining required, no water wash required, less load on the deodorizer, high quality deodorizer distillate
Catalyst Filtration	Remove nickel catalyst and colloidal particles	Less labor requirements, less usage of filter aid Less neutral oil loss, less solid waste creation
Solvent removal	Solvent recovery for reuse	Less energy usage, simple separation
Hexane vapor recovery	Remove hexane from air	Comply with clean air act bill, less hexane emissions
Miscella Filtration	Remove particulate	Better quality lecithin from degumming, less Centrifuge maintenance
Lecithin fractionation	Purify lecithin	Produce pharmaceutical grade products, better products and higher values
Air Purification	Remove hexane or dirt from Stripper bottoms	Produce clean air. Comply with governmental Regulations

Table 3. Tests with Commercial Elements

Feed	Flux
Hexane	29 - 72
Miscella	4 - 40
Miscella Feed Addition	19.1 - 47.1
Miscella Concentration	9.5 - 17.9
Washing lecithin with hexane	88

Table 4. Tests with Commercial elements

Tests	Flux (lmh)
Concentration	16 - 36.9
Washing	27.4 - 51.8
Hexane wash	25.6 - 71.5
Concentration with feed addition	12.5 - 52.4
Concentration	6.0 - 10.7
Concentration with feed addition	12.5 - 46.5
Concentration	6.5 - 11.3
Concentration with feed addition	11.9 - 48.3
Concentration	7.7 - 11.9
Washing lecithin	11.9 - 24.4
Concentration with feed addition	12.5 - 44.1
Concentration	6.6 - 10.1
Washing	19.7 - 21.4
Clean hexane	50.7 - 90.6

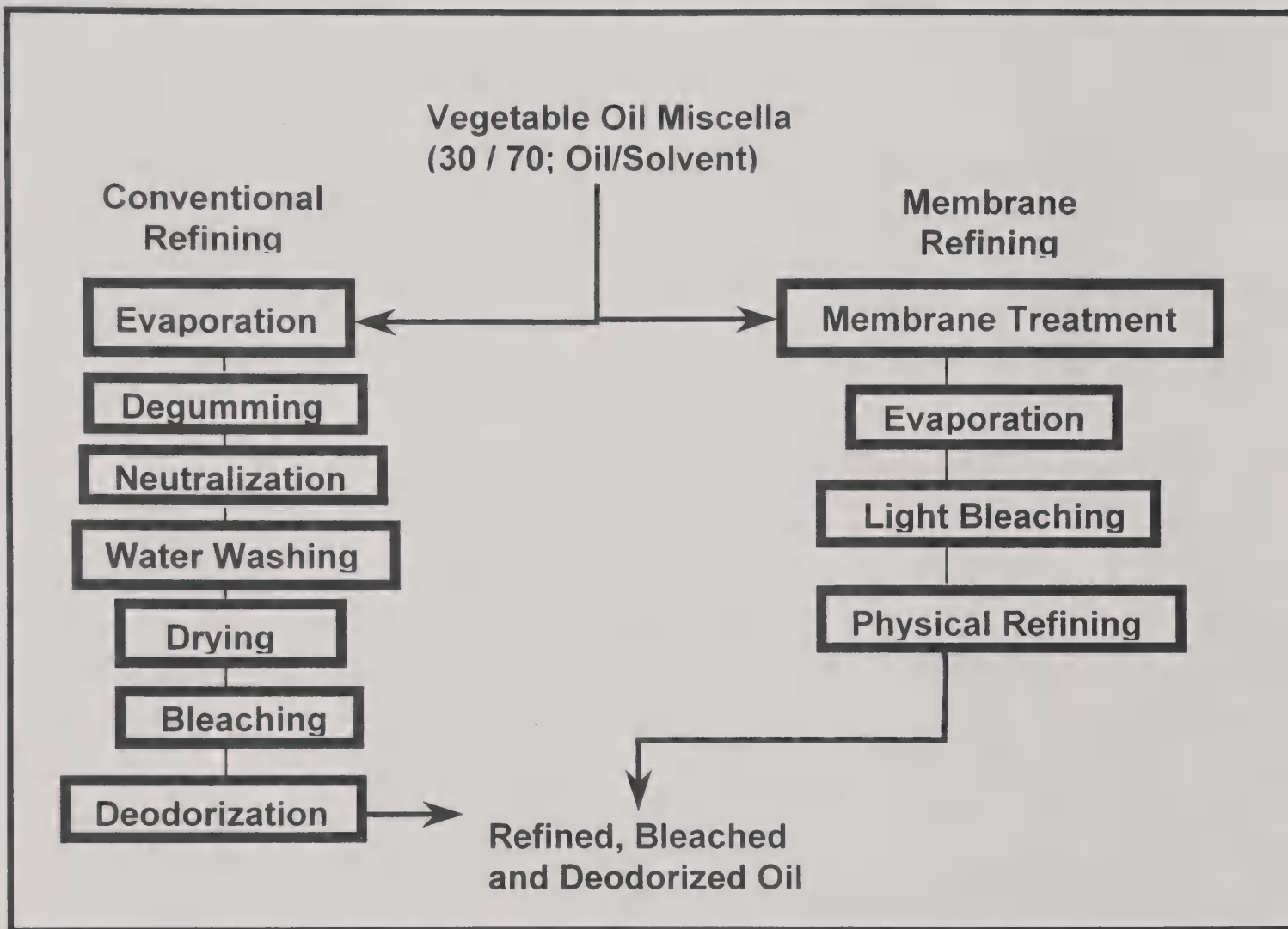


Figure 1. Flow Diagram of Conventional and Membrane Degumming Processes

REFERENCES

- Carr, R.A., ARefining and Degumming Systems for Edible Fats and Oils@, *J. Am. Oil Chem. Soc.* 55:765-771 (1978).
- Hoffman, G., *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products*, Academic Press Ltd., San Diego, CA, 1989.
- Jones, L.A., and C.C. King, *Cottonseed Oil*, National Cottonseed Products Association, Inc. and The Cotton Foundation, Memphis, TN, 1990.
- Koseoglu, S.S., K.C. Rhee and R.F. Wilson Proceedings of World Conference and Exhibition on Oilseed and Edible Oil Processing: Emerging Technologies, Current Practices, Quality Control, Technology Transfer and Environmental Issues Editors S. S. Koseoglu, K.C. Rhee and R.F. Wilson, AOCS, Champaign, IL 1997
- Koseoglu, S.S., Use of Membrane Technology in Edible Oil Processing: Current Status and Future Prospects, PORIM International Palm Oil Conference, September 23-28, 1996, Kuala Lumpur, Malaysia (1996).
- Koseoglu, S.S., Membrane Technology for Edible Oil Refining, *Oils & Fats International*. 5:16-21 (1991).
- Koseoglu, S.S., and D.E. Engelgau, Membrane Applications and Research in the Edible Oil Industry: An Assessment, *J. Am. Oil Chem. Soc.* 67:239-249 (1990).
- Lin, L., K.C. Rhee, and S.S. Koseoglu Bench-scale Membrane Degumming of Crude Vegetable Oil: Process Optimization, *J. Membrane. Science*. 3502 p.1-8 (1997)
- List, G.R., Vegetable Oil Degumming, in *Handbook of Soy Oil Processing and Utilization*, edited by D.R. Erikson, E.H. Pryde, O.L. Brekke, T.L. Mounts, and R.A. Falb, American Oil Chemists Society, Champaign, IL, 1980, pp. 362-365.
- Morgan, N.R., and S. Sanford, *Baileys Industrial Oil and Fat Products*, Vol. 1, 5th ed., John Wiley & Sons, New York, 1989, pp. 46-50.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Strategic Plans for Federal Role in Advancing Biobased Products

**Howard N. Rosen
USDA Forest Service
Washington, DC**

Key Points:

- **Major Federal Government effort to triple use of biobased products by 2010.**
- **USDA and DOE joining forces in this effort.**
- **Need support in Congress for new programs.**



Strategic Plans for Federal Role in Advancing Biobased Products

Howard N. Rosen
Staff Specialist, Biomass and Energy
USDA Forest Service
Resource Valuation and Use Research
P.O. Box 96090
Washington, D.C. 20090-6090
202-205-1557
hrosen/wo@fs.fed.us

Abstract

A major effort is underway in the Federal Government to triple the use of biobased products and bioenergy by the year 2010. The President has issued Executive Order (E.O.) 13134 to stimulate the creation and early adoption of technologies needed to make biobased products and bioenergy cost-competitive in large national and international markets. The Congress, concerned about energy security, environmental issues, and effective use of our natural resources, has introduced several bills consistent with Presidential interest in expanding the use of our crop and forest biomass resources. The Department of Agriculture (USDA) and the Department of Energy (DOE) are cooperating in a major effort, including a large budget initiative, to accelerate the use of biobased technologies.

February 1, 2000

Presented as a paper at the 49th Oilseed Conference in New Orleans, Louisiana, March 20, 2000

This Report was prepared by U.S. Government employees on official time and is therefore in the public domain and not subject to copyright.

Introduction

Biomass from the farm and forest has been used by humans since recorded history for food, shelter, and energy. In fact the general uses have not changed much over the millennia, only the quality and quantity of use. For example, forest biomass provided heat energy for prehistoric people; whereas today biomass is converted to steam, then electricity to drive heat pumps, which heat our homes. The United States, with rich farmland and abundant forest resources, is in an excellent position compared to other nations of the world to increase its use of agricultural and forestry materials for biobased industrial products and processes.

Before proceeding it is important to define what a “biobased” product is for this paper. A biobased product in this report is defined as *a commercial or industrial product, other than food or feed, that utilizes biological products or renewable domestic agricultural (plant, animal, and marine) or forestry materials*. Energy products, such as ethanol and biodiesel, are included in this definition. This definition is the same as the one used in Executive Orders and Congressional bills discussed in this paper.

The sustained economic growth of the United States (U.S.) depends on having a secure raw material source for industrial production. Petroleum, today’s prevalent industrial feedstock, is neither sustainable nor environmentally friendly. Biobased products offer alternatives to petroleum and mineral-derived industrial products currently in the marketplace which may have negative environmental impacts. Biological crop and forest materials for the fiber industries are renewable over a short time frame. At the end of their life cycle, these materials are either recycled or allowed to return to the environment in an environmentally friendly manner .

For many years the Federal Government has conducted and provided outside funding for research and development in the use of crop and forestry materials for biobased industrial products and processes. More recently Federal funds have been appropriated to USDA to facilitate the transfer of USDA-developed technology and to commercialize industrial uses for agricultural materials. Traditional and new agricultural and forestry materials can provide renewable raw materials for manufacturing a broad range of chemical, energy, construction, fiber, composite, and other commercial products.

Recently the Federal Government has tried to accelerate the development and use of biobased products which convert crops, trees, and other biomass into a variety of fuels and products. This paper discusses several of the important approaches of both the Executive side (under the President) and Congressional side of the government and attempts to get a cohesive National strategy.

Background

In recent years several programs and projects have been organized by the Federal government to encourage the development of a stronger biobased products industry in the U.S. A few of the major ones are discussed below.

The USDA Biobased Products Coordination Council (BPCC) has been in existence since September 1995 and was formed to share information, implement strategic planning, and provide policy advice to the Secretary of Agriculture on the Federal Governments's role in the development and commercialization of biobased industrial products from agricultural and forestry resources. The Council is chaired by the Under Secretary for Research, Education and Economics (presently Dr. Miley Gonzalez). Membership includes the following USDA Offices and Agencies:

- Agricultural Marketing Service
- Agricultural Research Service
- Cooperative State Research, Education, and Extension Service
- Departmental Administration
- Economic Research Service
- Foreign Agricultural Service
- Forest Service
- Natural Resources Conservation Service
- Office of Energy Policy and New Uses
- Rural Business-Cooperative Service

*The National Research Council Report, Biobased Industrial Products*¹, was sanctioned by the Board of Biology of the National Academy of Sciences, and prepared by distinguished scientists who formed the Committee on Biobased Industrial Products. The report was funded by USDA, DOE, and the National Science Foundation. The Committee concluded that "Federal support of research on biobased industrial products can be an effective means to improve competitiveness of biobased feedstocks and processing technologies, as well as diversify the nation's industrial base of raw materials,...."¹ The report provides many recommendations for specific research priorities.

E.O. 13101, Greening the Government Through Waste Prevention, Recycling and Federal Acquisition,² September 16, 1998 requests active involvement by USDA. That involvement requires the BPCC, in consultation with the Federal Environmental Executive, to prepare a list of preferred biobased products that will be part of an affirmative procurement program for all Federal Agencies.

DOE developed a Plant/Crop-Based Renewable Resources 2020 Program in 1998 with USDA input. This program calls for increased use of America's crops, trees, and agricultural waste for making a wide range of consumer goods such as plastics, paints, and adhesives. Specific

objectives of this program have been published in a document that outlines roadmaps and targets for the development and use of new crops and processes to make new biobased products.³ This program compliments Agenda 2020 for the forest, wood, and paper industry, a similar program which was started in 1994.⁴

A *BPCC-sponsored Retreat* was held October, 13, 1998 at the Patuxent Wildlife Visitor Center in Laurel, Maryland. About 75 key people from government, private sector, and university organizations met to identify ways in which USDA can be more successful in increasing the development and commercialization of biobased industrial products from agriculture and forestry resources. A strategic plan⁵ was developed from this meeting with consensus from the attendees. The plan directs the BPCC to focus its efforts over the next 5 years to increase the domestic research, development and commercialization of biobased industrial and commercial products by:

- providing USDA leadership in the Federal Government for increasing research, development, and commercialization of biobased products.
- increasing USDA's commitment to biobased products.

This strategic plan outlines USDA's approach to accelerate such development and promote the use of biobased products, such as increasing government purchasing of biobased products, a larger outreach on biobased products at national meetings, and increasing funding for biobased products programs in USDA.

Administrative Approaches

On August 12, 1999, the promotion of biobased products received a significant boost When President William Clinton signed *E.O. 13134, Developing and Promoting Biobased Products and Bioenergy*.⁶ This E.O. directs the further development of a comprehensive national strategy that includes research, development, and private sector incentives to stimulate the creation and early adoption of technologies needed to make biobased products and bioenergy cost-competitive in national and international markets. The signing of E.O. 13134 was held at the USDA with several hundred government, industrial, and university supporters in attendance. Those attending included the heads of the Department of Energy, Department of Agriculture, and Environmental Protection Agency; the Federal Environmental Executive; and Senator Richard Lugar of Indiana, Chair of the Senate Committee on Agriculture, Nutrition and Forestry.

The accompanying Presidential Memorandum challenged the Secretaries of Agriculture and Energy to develop options for the United States to triple the use of biobased products and bioenergy by the year 2010. E.O. 13134 creates several ways to coordinate this effort by establishing:

- an Interagency Council on Biobased Products and Bioenergy with the highest level officials to coordinate inter-government efforts.
- an Advisory Committee on Biobased Products and Bioenergy of 20 members from outside government to provide information and advice for consideration by the Interagency Council.
- a National Biobased Products and Bioenergy Coordination Office to ensure effective day-to-day coordination to implement strategic plans and to provide for the administrative needs of the Committee and the Council.

A budget initiative for Fiscal Year 2001 is critical to implementing E.O. 13134. This initiative allows the Department of Energy to concentrate on integrated systems for processing feedstocks simultaneously into a variety of products such as fuels, chemicals, and electricity and USDA to focus on increasing the economic viability for farmers and foresters to grow biomass products, develop new uses for biobased materials, and provide incentives to use bioenergy. The details of this initiative are currently under development.

Congressional Approaches

Three important bills have recently been introduced in Congress. These bills are consistent with the President's interest in biobased products and bioenergy.

*Senate Bill S-935, National Sustainable Fuels and Chemicals Act of 1999,*⁷ was introduced by Senator Richard Lugar following the publication of his article in *Foreign Affairs* in which he expresses his concerns on U.S. energy security.⁸ The article suggests that the use of biobased fuels could reduce U.S. dependence on foreign oil. S-935 establishes a board and an advisory committee similar to those recommended in the President's E.O. 13134. It creates a sustainable fuels and chemicals research initiative under a competitive process to carry out research on biobased industrial products. The bill Authorizes \$49,000,000 for each of fiscal years 2000 to 2005 for this competitive research and another \$14,000,000 each year to construct a USDA corn-based ethanol pilot plant.

*House of Representatives Bill HR-2819, Biomass Research and Development Act of 1999,*⁹ was introduced by Congressman Mark Uddall of Colorado and is similar to S-935. Differences occur in technical specificity, emphasis on basic research, and the lack of authorization for a USDA pilot plant.

*House of Representatives Bill HR-2827, National Sustainable Fuels and Chemicals Act of 1999,*¹⁰ was introduced by Congressman John Ewing of Illinois and closely follows S-935.

Future Plans

The Federal Government will be involved in many ways to implement E.O. 13134 and work with Congress on the Bills that have been introduced which support this E.O. Reports are being finalized outlining and assessing options for modifying existing programs to promote biobased products and bioenergy, as well as describing outreach effort to raise the nation's awareness of biobased products and technologies. The agencies are now working towards filling the various committees and councils to provide a coordinated effort in promoting and developing biobased products and bioenergy. It is important for those interested in the Federal efforts to promote biobased products and bioenergy to let the Congressional committees, especially those Appropriation Subcommittees on Agriculture and on Interior and Related Agencies know of your support for the Biobased Products and Bioenergy Budget Initiative.

References

1. Committee on Biobased Industrial Products. 1999. Biobased Industrial Products: Priorities for Research and Commercialization. National Academy Press, Washington, D.C., 123 pp.
2. Clinton, W.J. 1998. Executive Order 13101, Greening the Government Through Waste Prevention, Recycling, and Federal Acquisition. *Federal Register*, September 16, 1998. Vol. 63, (179):49643-49651.
3. Renewables Vision 2020 Executive Steering Committee. 1999. Technology Roadmap for Plant/Crop-Based Renewable Resources. DOE/GO-10099-706, Office of Industrial Technologies, Washington, D.C., 41 pp.
4. American Forest and Paper Association. 1999. Agenda 2020, The path forward: An implementation plan. American Forest and Paper Association, Washington, D.C., 31 pp.
5. Biobased Products Coordination Council. 1999. Strategic Direction for Biobased Products Work in USDA Through the Biobased Products Coordination Council. USDA, Washington, D.C., 6 pp.
6. Clinton, W.J. 1999. Executive Order 13134, Developing and Promoting Biobased Products and Bioenergy. *Federal Register*, August 16, 1999. Vol. 64, (157):44637-44642.
7. Lugar, R.G., et. al. 1999. Senate Bill 935, National Sustainable Fuels and Chemicals Act of 1999. Congressional Record, April 30, 1999. Washington, D.C.
8. Lugar, R.G. and Woolsey, R.J. 1999. The New Petroleum, *Foreign Affairs*, Vol. 78(1): 88-102.
9. Uddall, M., et. al. 1999. House of Representatives Bill 2819, Biomass Research and Development Act of 1999. Congressional Record, September 8, 1999. Washington, D.C.
10. Ewing T.W., and Shimkus J. 1999. House of Representatives Bill 2827, National Sustainable Fuels and Chemicals Act of 1999. Congressional Record, September 9, 1999. Washington D.C.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19-21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Down the Road to Value Added

Wayne Martin
PYCO Industries
Lubbock, TX



Wayne Martin
49th Oilseed Conference
New Orleans, Louisiana
March 21, 2000

DOWN THE ROAD TO VALUE ADDED

For those of you expecting a magic formula for success in adding value to your products, you are forewarned that you will probably leave this session disappointed. I do not have a magic wand that you can wave and add value to your product. Unfortunately there are no Interstate highways on the map leading to value added. In fact you may need to draw your own map.

To be successful on your journey down the road to value added there are several items you must give careful consideration. Some of those I will attempt to address with you today. Being a pilot I believe in checklist. So I prepared a checklist for us to follow as we prepare for this trip. You can call it developing a plan if you like.

1. We need a destination.
2. We need a map to follow so we don't get lost.
3. We need a timetable for the trip.
4. We need to check our vehicle to be sure it is in condition to make the trip.
5. Access to adequate fuel for the trip – we need enough to make the trip with some reserve.
6. Last, but not least, some idea of what we are going to do when we arrive.

First item on the list is the destination. The name of our destination is "Value Added", but you must define "Value added".

You start by determining what you intend to add value to, and how. Does it require further processing and/or packaging? What is the strength and weakness of your product? Does the ultimate consumer share your view of the product? You know what moving the product to value added means to you, but do you have any idea how it benefits the consumer. What will motivate someone to buy your product over all the other alternatives out there? Some questions you should answer before beginning this trip are;

what is my value added product going to be; is there a need for the product? What is the market, both in price and volume; what kind of competition are you going to face; do you really know your product? Normally, I am not a proponent of consultants, but you probably need outside help to develop the answers to some of those questions.

That brings us to the second item on the list, the map. We have the destination marked, but we need to determine the best route, and whether or not intermediate stops are necessary or desirable. Can you add incremental value to your product in steps? Is it possible to enter the market with a smaller part of your production and grow the volume with the market? Does your product fit into an existing distribution network or will you be required to develop the distribution for the product?

You need a timetable. More than likely you are going to be making modifications to your plant. This takes time, usually more than we first imagine. Murphy's law is alive and well. Your efforts need to fit the time line in some logical sequence. It is counter-productive if you make a sale and are unable to deliver.

You need to take a good look at the vehicle you expect to take on this trip. Is it in good running order or does it need a few repairs. If it needs repair, accomplishing that before you begin the trip is better than having to make those repairs on the road. In other words does your company have the personnel required to do the job and is your present operation running smoothly so as not to distract you from reaching your destination.

Do you have the proper structure and the personnel to accomplish your mission? If changes in structure or personnel are needed, those changes are better made before, rather than during the trip. If you are not willing to make those changes, then you probably should not undertake the journey.

You need access to adequate fuel to make the trip, including some allowance for unforeseen detours you might encounter. Can you afford to make the trip? Do you have money for unforeseen problems? If you lack the capital necessary to complete the journey, you would be ill advised to begin the trip. If you are out of gas and lack the ability to obtain more, the wrecker shows up and hauls your vehicle away. The wrecking yard is full of vehicles unable to complete the trip or return to the original starting point.

We follow the checklist. We have employed outside help. We have researched our product and all of the competitive products available. We have made the necessary improvements to our plant and to our organization. Our product will benefit the consumer. We have arrived. The sign says, "Welcome to Value Added".

The country boy has hit the big city. The big city is a confusing maze of streets and the traffic is unbelievable. Where do we want to go in the city and which street will get us there. Where in this city does our product fit? What is our target market? Do we have a mass market or a niche market product? Hopefully we determined that before we embarked on this journey.

If your target is the high volume mass market, think of it as a mall. If you were opening up a new mall what would you need to make it successful? First thing would be an anchor store, right. Do you know many successful malls without an anchor store? The anchor store in a mall draws in people. If you have a good anchor store, other businesses want to open in your mall. The anchor store sustains the mall and gives it the credibility necessary to attract other business.

If your market is the high volume mass market, your value-added product is no different. You need that large anchor customer to give you and your product credibility and to sustain you while you build the business.

If your product fits the specialty market, my advice would be to research the possibility of selling it online. My prediction is that something "Dot Com" will in time replace the "Chicago Board of Trade" and the "New York Stock Exchange" as we know them. Technology is the engine driving our economy. You should look for ways to use it to add value to your products.

In conclusion, let me encourage you to take the trip. It like any journey has some risk, but it also can be exciting and rewarding.

1000
1000
1000
1000

1000 1000 1000 1000 1000
1000 1000 1000 1000 1000

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Overview of EPA Vegetable Oil MACT Rule—Update on Major Provisions

Robert L. Ajax
Robert L. Ajax & Associates
Apex, NC

Key Points:

- **A new EPA MACT* rule will limit vegetable oil plant hazardous air emissions (e.g., n-hexane). *based on "Maximum Achievable Control Technology"**
- **The MACT rule includes stringent, not-to-be-exceeded numerical emission limits.**
- **Continuous compliance determinations, recordkeeping, and compliance status reports are required.**



Overview of EPA Vegetable Oil MACT Rule

Update on Major Provisions
(as of January 20, 2000)

Robert L. Ajax, P.E.

Presented to:
AOCS/NCPA Conference

3/21/2000

NOTES:

Broad Perspective -- MACT vs Other State/EPA Rules

- Clean Air Act of 1990
- State Implementation Plans (e.g., VOC)
- NSPS (e.g., boilers)
- Maximum Achievable Control Technology (MACT) for HAPs
- Title V Permits

NOTES:

Key MACT Rule Elements and Concepts

- Stringent not-to-exceed standards -- reflecting best demonstrated performance
- Rule requires continuous compliance
 - 12-"Operating-Month" rolling numerical performance limits
 - Operational Standards during startup, shutdown and malfunction (SSM)
 - Annual compliance certification
- Expect compliance to require best available equipment, and best operational practices at all times

NOTES:

Affected Seeds and Numerical Limits (12-Mo Rolling Avg's)

- Standards apply to HAPs (e.g., n-hexane)
 - Gal /Ton VOC limits listed below assume 64% HAP
 - Soybean 0.2 (Conv) 1.7/1.5* (Spec)
 - Dry Corn Milling 0.7 Flax 0.6
 - Cottonseed Lg 0.5/0.4*, Sm 0.7/0.4
 - Peanut 1.2/0.7* Canola 0.7/0.3*
 - Safflower 0.7 Sunflower 0.4/0.3*
 - Wet Corn Milling 0.4/0.3
- * Existing/New source stds

NOTES:

Startup/Shutdown and Compliance Plans

- SSM Plans
- Compliance Plans
 - Solvent Loss Measurement and Logs
 - + Malfunction Adjustment
 - + Daily inventories not required, but essential
 - + Shutdown at end of a month
 - Crush Measurement and Logs

NOTES:

Example Large Cottonseed Plant Monthly & 12 Mo Rolling Averages

Peak caused by tank measurement during shutdown does not average out; it causes a "blip" 12 months later

Possibly malfunctions -excludable if meeting stringent rule definition and SSM plans implemented



Aim for performance at least 0.1 gal/ton below the level exhibited here to ensure compliance

A non-operating month; Determine compliance with last 12 operating months

Probably a tank measurement during shutdown - Should not occur under the rule

Mo Ave

12 Mo Rolling Avg

CK95

Compliance Schedule -- Starts on Promulgation (Est ~ 12/00)

- Existing Source Dates (See Rule for New/Reconstructed Source Dates)
- 120 Days -- Submit initial notification
- 3 yrs -- compliance begins
 - SSM and Compliance Plans completed; Implement these plans as needed
 - Begin collecting/recording data
- 3 yrs, plus 12 operating mo, plus 1 month (generally 49th mo)- compute compliance
- 2nd month after 12 operating months, submit initial compliance report

NOTES:

Other Notifications/Reports

- Exceedance Report due within 30 days after any 12-month period numerical standard was exceeded
- SSM reports due within 30 days after any month SSM was triggered and SSM plans were adhered to
- Report within 2 days of any SSM election, and there were deviations from SSM plans

NOTES:

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Specialty Oils Outlook—New Realities

Bill Soucie
Protein Technologies International
St. Louis, MO

Key Points:

- **Agricultural biotechnology will need to be consumer driven in order to achieve maximum success.**
- **Consumers will not accept new technology unless the benefits outweigh the perceived risks.**
- **Oilseeds will continue to play a key role in bringing health and nutrition to consumers.**



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19-21, 2000

New Orleans, LA

Title: Specialty Oils Outlook – New Realities

Bill Soucie, Ph.D.

Protein Technologies Int'l

St. Louis, MO

New realities for specialty oils are principally driven by the impact of biotechnology on agriculture. This impact of biotechnology has shifted over the years and in many ways the global issues surrounding biotechnology will buffet the progress of specialty oils in the marketplace. As used in this paper, biotechnology refers narrowly to gene transformation or genetically modified organisms (GMO) rather than to the broader definition that also includes traditional mutagenic and breeding methodologies. The terms biotechnology and GMO will be used interchangeably in this discussion.

1990 View

In 1990, Biotechnology was embraced by the pharmaceutical industry. Companies succeeded in bringing new drugs to the market, for example, human growth hormone and insulin, using gene transformation systems. The benefits to the consumer were clear and “GMO” was not an issue.

During this time, many genetic engineering start-up companies were looking for venture capital in the food industry. Much of the technology was focused on manipulating enzymes to alter biosynthetic pathways for vitamins, protein, carbohydrates and oils. Progress was slow because the cost of entry was too high for the modest margins achievable in food markets. Although the gene technology was an intriguing tool, the marketable consumer benefits were difficult to define.

There was little consumer awareness of biotechnology during this time period, but generally biotechnology was presented favorably and was considered the wave of the future in modern biological science.

Identity preserved (IP) production was still at low levels and primarily for field testing for regulatory approval. No commercial GMO food products were in the market in 1990.

2000 View

By the year 2000, billions of dollars have been invested in biotechnology including in the food and agriculture industries. Small pharmaceutical start-up companies have either grown into large businesses or other companies have purchased them. Some of these start-ups like Genentec and Amgen have grown into major pharmaceutical companies, thus demonstrating the power of this new biological tool. The primary

focus of biotechnology in agriculture has been on input traits such as disease and herbicide resistance of crops.

The cost of biotechnology for food applications is still significant but the cost is now in a range where many food companies are trying to discover opportunities to use biotechnology in their processes and products. A challenge in agriculture has been to find consumer benefits (output traits) that are of interest to consumers and that can be delivered by biotechnology. Calgene was the first to offer a consumer benefit, namely vine-ripe flavor in a store bought tomato. The tomato has had mixed success in the marketplace. Monsanto/Calgene are also marketing high lauric acid canola oil that is a GMO product. Protein Technologies International, a Dumont business, has high oleic soybean oil, which has been approved by the FDA for use in food. This oil is in advanced stages of market development. The vegetable oil is very stable and will help food processors to reduce the content of trans fatty acids in food products. Other than these three examples there are no other GMO-based products in the market that directly deliver consumer benefits. There are, however, many products under development and in the regulatory approval process.

Consumer awareness of biotechnology has increased but still is not as high as some would think and in fact, there has been some erosion in awareness. This erosion is surprising in the face of recent news coverage of anti-biotech special interest groups. For example the Wirthlin Group Quorum Surveys conducted on behalf of the International Food Information Council in 1997 and 1999 showed the following:

Total consumer awareness of biotechnology:	1997 – 79%
	1999 – 69%

The numbers are smaller if we only consider consumers that have heard or read “a lot” or “some” about biotechnology:

	<u>1997</u>	<u>1999</u>
A Lot(%)	11	7
Some(%)	35	26

Another section of the survey showed in 1999 that only about 5% of consumers feel that they are very well informed about biotechnology.

Compared to 1990 there currently has been controversy around biotechnology, primarily driven by special interest groups in Europe in response to the entry of herbicide resistant soybeans and corn. These issues are now influencing some U.S. based food companies as well. The result of this controversy is that there will be food-labeling legislation that requires labeling of GMO products and also, there will be markets for GMO and non-GMO products.

By 2000 there has been significant experience in IP production for crops although most are not GMO. For example, several oil products are in the market such as low linolenic and low saturate soybean oil, high oleic and low linolenic canola, high oleic sunflower and high oleic safflower and flax. These oil products deliver health and functional benefits and all are produced using IP production in order to maintain their identity from their commodity counterparts. Some difficulties have been experienced in adapting commodity-processing systems to meet the requirements of IP production. A key issue has been that the volume of production required for specialty oilseeds is usually far below the minimum capacity of the large oilseed mills. As IP production increase this limitation should correct itself. This author is not aware of the total number of IP acres for oilseed production in North America, but there are likely 1-2 million acres.

There is still a very high interest in using biotechnology in food processing but cost is still a challenge and the delivery of consumer benefits rather than productivity gains still needs to be increased. In oilseeds we can anticipate seeing new oils with high saturated fatty acids, elevated omega-3 fatty acids, and elevated antioxidants. New proteins will also be available with enhanced functional properties as well as with enhanced isoflavone content. Many of these traits will be combined within the same oilseed in order reduce costs.

New Realities

The Anti-GMO sentiment that became extreme in Europe and is affecting business in the U.S., Japan and other countries. This is a clear demonstration of the global aspects of the food business and certainly of the oilseed business. Also, the current situation in Europe has shown that the political climate can override the regulatory approval process. There is general agreement that biotechnology is safe and that regulators are using science-based decision making to regulate products; however, political concerns are now interfering with this process.

IP production was originally developed to preserve the consumer benefit of modified crops from being diluted or lost by mixing with the comparable commodities. Now, IP production has several purposes:

- preserve the consumer benefit (output trait)
- traceability
- food safety
- produce non-GMO crops

Food processors are now interested in traceability of their ingredients as well as having a heightened awareness of food safety. Many companies are asking for non-GMO ingredients. All of these features available through IP production are of interest to food companies to help meet the demands of their customers.

Food companies are now required to obtain regulatory approval for biotechnology-based products in many countries due to the multinational aspect of their businesses. Consumers also have different needs and desires in different countries – “one size fits all” does not work in most food products.

Ironically, we have created a whole new segment: non-GMO. In many cases the non-GMO product is the original commodity product. Based on the current climate around biotechnology this non-GMO segment may exist for several years.

What have we learned?

1. Agricultural biotechnology will need to be consumer driven to be successful.
2. If biotechnology is used to deliver benefits that are clear to consumers, the technology will be accepted.
3. Consumers will not accept new technology unless the benefits outweigh the perceived risks.
4. All providers of biotechnology and biotechnology-based products need to respect consumers' concerns and desires.
5. As IP production increases it will put strains on the commodity production system. It will take a coordinated effort by all stakeholders in the oilseed production and processing industry to successfully develop large-scale IP production systems.
6. Oilseeds (both protein and oil) will continue to be central in delivering consumer benefits, especially from a health standpoint. Biotechnology will play a major role in creating these new oils and proteins.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Leadership and the Changing Work Environment

**Joan L. Bicocchi
J B Consulting
Joan L. Bicocchi & Associates
New Orleans, LA**

Key Points:

- The amount of legislation and the number of classes protected under the anti-discrimination laws will continue to expand.
- The key qualities that will result in the ultimate triumph of the Information Age are: visioning, globalizing, decentralizing, empowering, and harmonizing.
- Leaders will have to learn new competencies related to these qualities to be successful in the new millennium.



LEADERSHIP
and
THE CHANGING WORK ENVIRONMENT

Presented by:
Joan L. Bicocchi

J B CONSULTING
Joan L. Bicocchi & Assoc.
3630 Rue Nichole
New Orleans, LA 70131
504-393-0630
504-433-0630
jbconsult@compuserve.com

Expanding Employment Law- Over the years employers have heard lecture after lecture about their obligation to refrain from discrimination. Some illegal practices have become rather obvious such as: advertising for applicants aged 18-30; inquiring if applicants are married or have small children; stipulating different qualifications for the same job depending on color or gender of who applies. While awareness and knowledge of Title VII of the Civil Rights Act has grown since its original enactment in 1964, so has the amount of legislation and the number of classes protected under the anti-discrimination laws. There is risk of violating the law when a person or a group is treated differently for reasons that do not serve a valid business purpose. A particular form of discrimination is illegal when Congress, a state legislature or a city council decides that a characteristic (race, ethnic group, color, gender, religious beliefs, national origin, physical disability, or age if a person is at least 40 years old) bears no legitimate relationship to employment decisions.

Other types of discrimination are more subtle but just as illegal. Employment practices having a disproportionate and discriminatory impact on certain groups are also barred by anti-discrimination laws. For example, if a primary means of recruiting is through employee referral and a company workforce consists entirely of Caucasian males, the practice could be discriminatory, that is, if the effect perpetuates the same workforce race and gender composition.

In the last few years, age discrimination has become more prominent as companies restructure and downsize older workers. Replacing an older employee with one 40 years old will not insulate an employer from discrimination. Although both are in the protected age group, the fact that a replacement is substantially younger is a far more reliable indicator of age discrimination under the Americans with Disabilities Act (ADA).

The new head of the Equal Employment Opportunity Commission, Ida Castro is an outspoken critic of pay inequities between men and women. She intends for the agency to be more out front on wage

discrimination issues, understanding that they are key to the economic progress of significant sectors of our society.

The Changing Environment- The future work force management is to become increasingly complex. There is a dynamic relationship between strategy, people, technology and the processes that drive organizations. As employers tap the talent pools overseas, the work force will become increasingly diverse. The diverse composition of the work force means employers will have to ensure that they reward effort, not prejudices against sex, race, age, national origin or other global differences. Public pressure will intensify for companies to be good global citizens and to restrain from new investments in places that permit unacceptable employment practices.

More and more classes of employees will receive protection under discrimination statutes in the years ahead. The ultimate inquiry involves a determination of fairness. Juries will not return a verdict for the employer unless they are satisfied that the company acted in good faith and was fair in its dealings with the employee/plaintiff.

The Industrial Age brought us the economic power and conveniences we enjoy today. Now we have entered a new age...one that is characterized by so much change most of us can scarcely keep up with what kind of phone to purchase. The new era, the Information Age will be characterized by more change. Learning and resiliency will be paramount to achieving success.

Changing from Management to Leadership- Consider for example the feature article that appeared in Fortune magazine in February 1993. Fortune's cover had headlines that read, "Dinosaurs?. The decline of these 3 giants is the biggest what went wrong story in US business history. There are lessons here for everyone." The article points out that in the 1990s the three giants (IBM, Sears Roebuck, GM) went lame or suffered an industrial accident (so to speak) in remarkably similar ways and in a manner that provides pointed lessons for all of business. What were the causes for their decline at that point in time? Arrogance and bureaucracy were primary! Arrogance and bureaucracy had crept into their organizations and inadvertently they devised ways to fail through their arrogance and bureaucracy. Their onetime leaders didn't keep up, reports Fortune. Companies don't stumble; people in them do. As Peter Drucker has said, "Every failure is a failure of a manager."

Jack F. Welch, Jr., CEO of General Electric is one of the few top leaders who has continued to keep GE in the top 10 since the 70s. In his 1991 annual report to stakeholders he writes, "There is a type of leader that is the most difficult to deal with. That leader delivers on commitments, makes all the numbers, but doesn't share the values we must have. This is the individual who typically forces performance out of people rather than inspires it: the autocrat, the big shot, the tyrant. Too often all of us have looked the other way. But now these people must go."

Welch's letter is clear. The management style of 1900's is to be replaced with a new style of leadership. To avoid becoming dinosaurs, company leaders must lead differently and people work together differently. Rosabeth Kanter in her recent article in Inc. Magazine refers to this new way of working as the "E-culture--the human side of the global information era, the heart and soul of the new economy." To take full advantage, if not to survive, requires challenging traditional models of organization, communication, decision making and of leadership.

The old command and control model is becoming more and more outdated. Why? Because it can't work any more...not when no one person in a company or even on a team can have all the knowledge that's needed to make quick responsible decisions. The pace required for efficiency is too fast, the information for effectiveness too new. In the past, a manager was expected to know all there was to know about his area of work; today that is impossible. Entering this 21st century, leaders will have to rely increasingly on their employees to make and to execute good decisions rapidly.

Globalizing and decentralizing- Technology, especially the Internet is leading us to globalization. Where leaders in the past may have inherited positions at the top of a ladder, leaders of tomorrow will struggle with how to dismantle those inherited hierarchical building blocks and reassemble them as global webs. E-business thrives on the strength of networks, on the connections among the entire extended business relationships. As geographical boundaries dissolve, companies need to reach markets quickly, Alliances and partnerships become a powerful source of advantage. Very small companies gain clout by being linked to wider networks. Centralization cannot exist in a web. In a web, power is diffuse and resides closer to those who directly face the currents of change. For a network to function properly, all nodes must be healthy, resilient, and capable of coordinated but independent action. In a network

every action affects all players, every loss or gain is seismically transmitted to all parts. It's similar to a spider web that when tapped, instantly radiates signals through the miraculous fibers. Leadership is in networks. Business leaders must become ambassadors beyond the company walls and employees diplomats. Where leaders in past times may have hoarded information, today they must learn to spread information. Playing "I've got a secret" is no longer a way to feel powerful. Networks involve the art of dialogue, testing, negotiating, boundary breaking yet always with a shared purpose and values.

Empowering and Building Leaders- If you want to grow and succeed in this new millennium. Jim Belasco and Ralph Stayer in their book, **Flight of the Buffalo** identify that leaders' biggest challenge is to get the buffalo (a word they use synonymously for leader) to learn to fly. A loyal herd of buffalo waits for the leader to tell them what to do. However, if you ever notice a group of geese flying in their "V" formation, you might know they change leadership frequently, with different geese taking the lead. They alternate between being a leader, a follower or a scout."

They emphasize that organizational leaders can only do this by learning to let others lead. This is not abdication, however, it is to stop being the center of the organization and to let the vision and customer be the center. The leader then stops doing and helps others to do, helps others to become leaders in their own right. However, a leader cannot just one afternoon say, "You're responsible make your own decisions," that would be jumping from authoritarianism one day, to abdication the next. You cannot command the buffalo herd to fly suddenly.

You must empower people for a new level of performance. To those of you who have not liked the word "empowerment" applied to employees, employees have always been empowered, that is, they could always make or break a company. It's just that management has typically never wanted to admit it. "The best way to empower people," Belasco writes, "is to ask: what am I doing or not doing, as a leader, that prevents them from assuming responsibility and performing at the next level." One manager that Belasco has written about began to tape record meetings so that he could listen for whether he had restrained from making all the decisions.

Noel Tichy, author of the Business Week Book of the Year, *The Leadership Engine* states, "All people have untapped leadership potential, just as all people have untapped athletic potential. It

doesn't matter whether you are a senior executive or a part-time hourly worker in your organization. You have leadership potential that you aren't using. With coaching and practice, you can dramatically improve your ability to lead and to help others be leaders as well." *What is critical for success today and tomorrow are leaders capable of continually learning and teaching others to be leaders.*

Harmonizing- Disney World has recognized globalization since inception of its theme park. When you or your children are there, you hear the song "It's a Small, Small World". It's a song that reflects the smallness of our planet and its diversity. The song is even more relevant today as it extends to groups of people of unknown composition as far as our e-mail can be forwarded.

Yet, beyond our obvious differences of racial, gender, nationality, religion, there are some common human characteristics that we all share. We all have a desire to be healthy, to have the love of family, to live comfortably, to perform meaningful work. Whether in a real organization as small as a family or in a virtual organization as large as our E-mail provider, finding the elements that link us, that provide the connection between us will become increasingly significant.

Until recently scientists operated from a mechanistic model. Now scientists are finding new ways to explain and describe the universe. Scientists now see that in the new framework of complexity, indeterminacy and chaos that everything participates in creation and we really don't have control over the impact that we have or anything else has. We do know that existence is a relationship thing. Nothing exists without being in relationship with something else. The old science focused on the parts rather than their relationships. The new science suggests we must focus on both. We are therefore co-creators of the future. Everything is interdependent. With this recognition more conscious responsible involvement is required. It demands integration of people. It moves us beyond independence and dependency to partnership and alliance.

"If previously, running a business was more like directing a play and reading a script, in tomorrow's environment, it is more like improvisational theater. The employee-actors are given a theme or a vision and they interact with one another to create products and services...creating ever improving versions using audience reactions," writes Rosabeth Kanter in last month's issue of Inc. magazine.

To adapt to our new circumstances--expanding markets, shrinking capital, foreign partners, constituent demands--leaders must learn the subtle competencies of embracing opposites, dealing with paradox, accepting ambiguity. Where before leaders saw competition, even conflict, as the natural route to glory, in the new millennium they are more than likely to craft collaboration. Consider for example the emphasis being placed on building alliances in today's marketplace. Texaco and Shell Oil, previous archrivals, created Equilon and Motiva a joint effort in the downstream oil and gas business. Many others could be identified if we had the time. Certainly there are economies of scale to be gained yet the concept was unheard of years ago. The idea here is to search for what unites us over that which separates us, to build something better for the benefit of both. Creating win-win agreements or finding ways to "harmonize" will have increasing value. Managers of yesterday were less concerned about harmonizing than the leaders of today and tomorrow can be if they are to succeed. Harmonizing is an art that requires people and personal effectiveness.

Learning and Thriving Through Change- Earlier I mentioned the Fortune article regarding becoming a dinosaur. You recall that managers become extinct through arrogance and complacency. The Chairman of Intel, Andy Grove refers to it as, "the inertia of success and it is very dangerous." New learning and resiliency is imperative to thrive through the revolutionary changes we are experiencing. The leader's worst enemy lies not "out there" but within the confines of old habits and beliefs. "You may prefer to hold on to the notion that the world is flat, but that doesn't make it true, and it certainly won't speed your ships journey to the Indies. In a world of blended cultures and contradictory values, leaders must learn to question, and occasionally abandon, comfortable notions of black and white thinking. It is not abandoning core values, but allowing others to be faithful to theirs. The prerequisite competencies for the leaders who will be successful are valuing others, empowering them, and harmonizing. In addition, leaders will have to possess a powerful, motivating vision of what is possible.

Visioning – Without vision, you have no proactive direction in which to organize the available resources. Vision allows you to create reality, not merely to react to it. Successful people and leaders begin their journey to success with a vision. Vision starts from the answer to a simple question: What do I really want? What do I most want for

this organization? Visionaries live in this question, asking it so frequently, they inspire others to start asking it. Questions of this nature are powerfully transformative. When you ask them in a genuine state of inquiry you get brand-new information from your creative mind. You begin to expand it by asking others.

“By believing passionately in something that still does not exist, we create it. The non-existent is whatever we have not sufficiently desired.” Nikos Kazantzakis

Visionaries know they cannot have everything. They know they must work on refining and narrowing their focus. Yet as Eleanor Roosevelt knew, “You must always do the thing you think you cannot do.” The question Robert Schuller poses is, “What would you attempt to do if you knew you could not fail?”

Visionary leaders’ passion and dedication have to be bigger than other people’s attachment to the way things have been, bigger than their fear of change and of losing control. A visionary leader stands in the future and looks back toward the present. The vision allows you to establish yourself comfortably in an imagined positive future. Yet, simultaneously be firmly in the present. Visionary leaders cultivate an ability to live in multiple time zones....all at the same time.

If your vision is strong and big enough, you’ll view barriers as challenges on the way to something grander rather than as obstacles. Walt Disney said, “If you can dream it, you can do it. Leaders know how to transplant themselves into the future and describe the steps necessary to get there. Creating tomorrow is easier than changing tomorrow. Reacting to change by restructuring and rightsizing will not determine the winners in this new century. Creating for tomorrow and then figuring out how to manage backwards is more what leadership is about. Consider two recent pioneers, so to speak, who embody this very thing. We have all heard the story of Bill Gates and Paul Allen. These 2 Seattle nerds in their twenties pooled \$50,000 to buy an existing piece of software that they tweaked and retitled Microsoft Disk Operating system (MS-DOS). These two men believed that the personal computer would become a common household item. A notion that IBM originally rejected as they held on to their very successful mainframe systems. They paid heavily for that inertia.

This is not to say that leaders do not face the realities of the external market and make realistic assessments of their capabilities. They

do! In addition they develop new ideas for their organization and design bold, innovative actions that display courage. In this way, they develop edge, both portfolio edge and people edge. Edge grows the business in the long run. The test of edge is the courage to see reality and act on it.

There was an article a few weeks ago in USA Today that exemplifies the new leadership. Motorola's stock dipped to \$38 a share in mid-1998. Eighteen months ago investors had just about given up on Motorola. Yet, look where it is now. It's share price is going for around \$140. How did a entrenched 170 year old company bounce back like that? "We gave up on the thought we could incrementally and frequently change things. It didn't work. We decided that we'd change absolutely everything, except our principles", said Chris Gavin, who was named CEO in January 1997. "One of our key new strategies was to realize we couldn't do everything ourselves. Because we cannot respond to changes rapidly enough, we had to focus our energy in the laser-like fashion that today's environment requires." Over the past year Gavin has struck key partnerships with Lucent Technologies, Sun Microsystems, and Cisco Systems. Actions unheard of for the once-insular Motorola! Another action underway is to set up an internal venture capital unit, modeled after Intel's VC unit. The risk Motorola believes is that "if we stop changing, we'll slide back."

Gavin demonstrates openness to changing, to learning. Motorola's top leader is introducing new ideas, decentralizing Motorola, empowering employees. By building alliances he is harmonizing. He is embracing key qualities that will result in the ultimate triumph of the Information Age: visioning, globalizing, decentralizing, empowering, and harmonizing.

As a leader, are you ready to embrace the new millennium?

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Consolidation to Build Branded, Global House

**Rod Smith
Feedstuffs Newspaper
Minnetonka, MN**



Consolidation to build branded, global house

■ By ROD SMITH

Feedstuffs Staff Editor

"Life on this Earth is not designed for the past" — David Kjome, University of Minnesota.

Agriculture, in the U.S. and worldwide, is both consolidating and integrating for one reason: It must.

In the future, agriculture and food production — from corn and soybean fields to dairy farms and dairies, to cattle ranches and feedlots, to hog operations, to poultry complexes, to packers and processors — will be consolidated into huge, integrated production systems. It will consolidate and integrate so that agriculture itself and independent, individual producers themselves can compete, expand and prosper.

Consolidation is already an established event. In the U.S., essentially five companies control most of the field seed business, 4-5 companies control most of the meat packing business, 20-

25 companies control most of the chicken business and 10 companies control half of the food retailing business. Within 10 years, 30 beef cattle feeding and 50 hog-producing businesses will finish 50% or more of all beef cattle and hogs.

Consolidation is documented and reported, consolidation is occurring in every industry in every country in the world, and consolidation is indisputable and irreversible.

Those who have sought to explain the phenomenon — at levels above clashing ideologies — have said there are certain critical points ushering agriculture consolidation, each leading to or supporting another. If one understands them, he or she will make the future.

Consumer demand:

Building a branded house

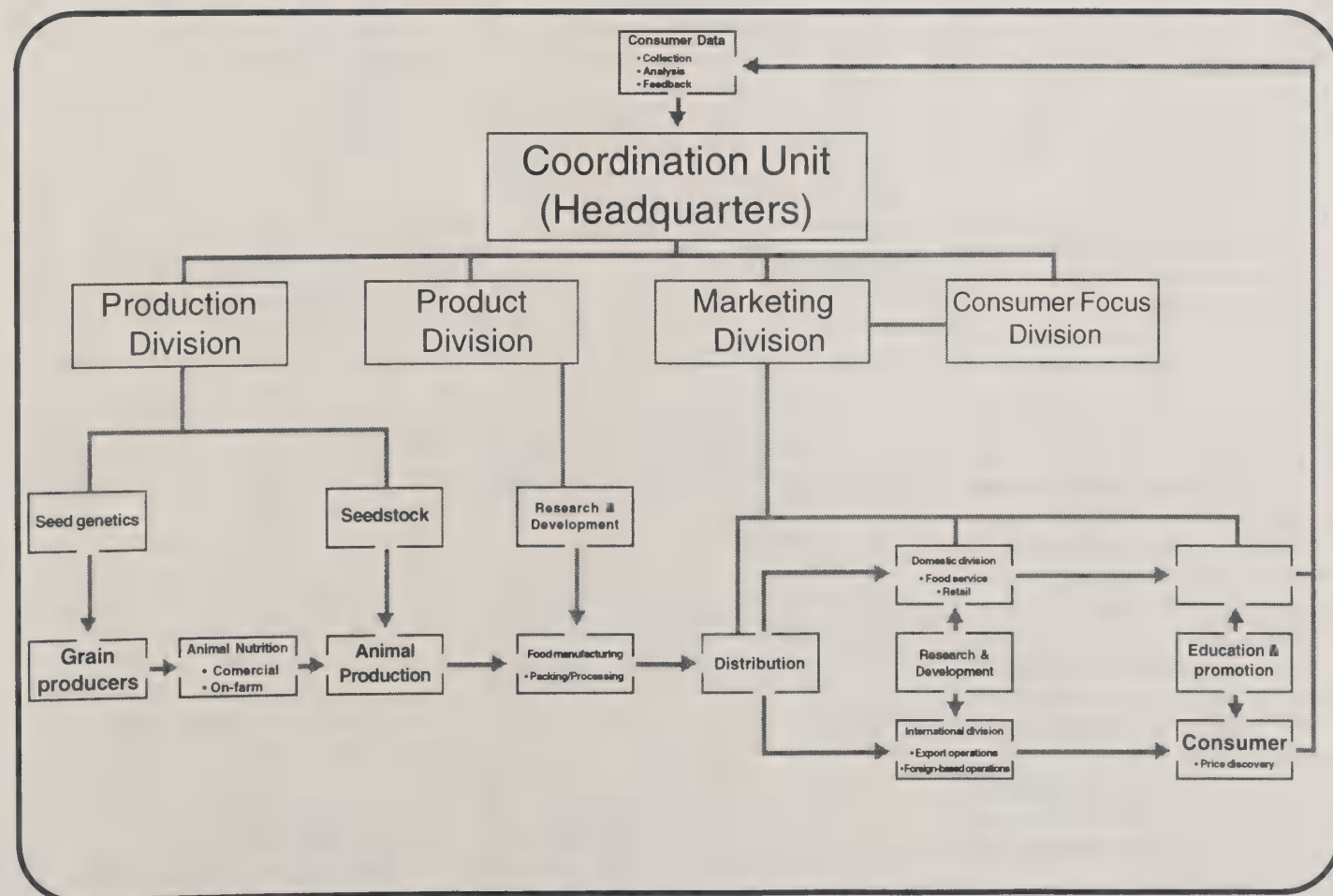
As with all food issues, consolidation starts at the end — with consumers and their requirements for their food. Con-

sumers demand food that's healthful, nutritious and safe, high in quality, tasty, convenient and a price value. Consumers demand food produced in an environmentally sustainable way, and if animal-derived, from animals that were humanely raised.

Consumers have little patience or time — or the knowledge — to sort through grocery stores and meat cases to find dairy, meat and poultry products that will meet their expectations. Accordingly, they are buying and will increasingly purchase brands because brands represent an assurance of the integrity of the products selected. Brands will be their guarantees.

Foodservice operations, then, will buy and serve brands that will be promoted in menus and other ways, and retailers will buy and merchandise brands. Brands will be their guarantees to their customers.

Foodservice operators and retailers



will demand brands, including beef and pork brands, from suppliers — labels that carry processors' names or even a restaurant's or store's name.

Processors, therefore, will develop, produce and promote brands, and that is where the stakes get high. Brands have a name on them. Brands say to the customer — the operator, the retailer, ultimately the consumer — that a product will deliver expectations every single time. If it doesn't, even just once, it risks the distrust of the marketplace and the loss of the market.

Processors must deliver absolute consistency in every box, in every truck, every day. Processors, therefore, will need consistency in manufacturing processes and raw materials, and in the dairy, meat and poultry sectors, they will procure raw materials from suppliers who can provide beef, pork, poultry and milk that, when further processed, become guaranteed products on which a brand, a name, can be put.

Packers and other suppliers, therefore, must deliver those raw materials every time. Accordingly, they will demand cattle off the feedlots, hogs and milk off the farms and eggs and poultry off the complexes that have the carcass qualities that deliver the raw materials that can be processed into the products that make the house that the brand builds.

Producers, therefore, must husband animals from genetics, animal health and nutrition programs and other production regimens — animals managed

to precise standards — for that brand, for that house.

All of this — from farm gates to further processing plants to consumers — requires a production system that can identify and “integrate” management strategies so that the finished product wins as a brand, in the marketplace, through to the consumer.

Global demand:

Building a worldwide house

The production system must also deliver the quantity required because a brand that's not available every time a consumer goes into a restaurant or retail store will be as fragile in the marketplace as one that fails to meet quality expectations. Consistency applies to both quality and quantity.

This is especially true considering globalization, in which democratization and freer trade have not only opened markets around the world, but have strengthened economies and increased demand for branded, western protein.

Here's another point where the stakes get high.

By 2050, the global population will have expanded from 5 billion people today to 10 billion people with more affluence and demand for beef, pork, poultry and dairy foods than ever before. However, populations in the traditional global markets will have hardly budged, while the Asian population will have expanded 52%, the African and Near East population 33% and the Latin American population 9%.

Clearly, for agriculture to expand, it must expand globally. Clearly, for beef cattle producers in Montana, dairy producers in Wisconsin, pork producers in Iowa and poultry producers in the Southeast to expand, they must access markets worldwide.

Certainly, 85% or more of all beef, pork and poultry production is consumed domestically and producers should be careful not to disregard that market, but expansion in that market will become increasingly inconsequential next to market opportunities worldwide. Production must have the brand and the scale to be worldwide.

However, from Montana to the Southeast, American producers are not alone. Globalization has not occurred only to benefit American agriculture (and other American industries), and agriculture elsewhere realizes, too, that the future requires brands and scale.

This is not a side statement. Agriculture elsewhere is competitive, with benchmarking data showing that Brazil, as one example, already can pro-

duce chicken, hogs, soybeans and other commodities as or more efficiently than the U.S. Agriculture from the Prairie provinces of Canada to the steppes of Eastern Europe can potentially compete with the U.S.

Furthermore, it's the brand consumers buy, not the country of origin, which means in the U.S. and worldwide, beef brands from the Argentine Pampas or pork brands from the Brazilian interior may become the brands of choice if they deliver the guarantees consumers demand.

Accordingly, agriculture, from farm gates to plants to consumers, requires production systems that are coordinated enough to develop brands and large enough to be global players.

Biotechnology, equity:

Building a strong house

To be branded and global, agriculture is consolidating into food production systems that will be few in number and very large, each consisting of thousands of producers, including corn and soybean growers and feed manufacturers, and packers and processors (Figure, p. 1). Each system will be focused on producing one or more brands following highly coordinated management and production “protocols.”

In the case of livestock or poultry, each system will be focused on one or more brands of beef, pork, poultry or dairy products, may be multi-species and may include non-animal foods in divisions, or “subsidiaries,” for fruit, produce, vegetables or spices.

Enabling, indeed speeding, the creation of these systems will be biotechnology and equity funding.

Biotechnology to now has been focused largely on input traits, i.e., how can this corn seed be genetically manipulated to grow faster, improve yields or resist drought or insect damage. That has been more or less a commodity orientation of the science, and although it would add efficiencies and price competitiveness to a production system, it does not add enough value to production to make the system a branded house.

However, now biotechnology is starting to focus on output traits, i.e., how can this corn seed be manipulated so that when its plant is harvested and processed into a feed, the feed will improve the performance of the animal so that the animal produces high-quality meat, milk or eggs? This is a value-added orientation of the science, permitting a system to add value to production and create its brand, or brands.

As for the equity issue, there is an in-

(EDITOR'S NOTE: Over the last 15 years, Feedstuffs Staff Editor Rod Smith has interviewed nearly 1,000 board chairpeople, chief executive and chief operating officers, strategic planners, analysts and consultants, economists, extension specialists, packers and processors, foodservice operators and retailers and producers themselves at their farms and ranches across the U.S.

(He has reported their views about how agriculture and the business of food production — from corn seed to meat, milk and eggs — will eventually and of necessity consolidate into large production systems so that American agriculture and individual producers can expand and succeed in a competitive, global marketplace.

(This article represents a kind of “consensus” of all those interviews and an analysis and interpretation. It is published this week as events in much of agriculture in the past year appear to have pushed the industry to a critical stage in the evolution and understanding of consolidation.)

creasing need for fund managers to identify new places in which to re-invest earnings from mutuals, pensions and other investments. These are accumulating massive returns as individual investors, not well-versed in the art of the market, are subscribing to the theory that they just let their profits run.

These fund managers are becoming increasingly interested in agribusiness and agrifood stocks, especially given acquisitions and mergers in those stocks that create critical mass and focused management, both essential to efficiency, market share and profits.

These fund managers also are becoming increasingly interested in production systems. Managers see that these systems will be so coordinated that production will closely mirror what can be sold so that there will be less exposure to commodity cycles; so coordinated that production will be consistently high in quality and targeted to a balance in demand and quantity, and so coordinated that production will have efficiencies, market shares, brands and global presence that will lead to profitability — to returns that will be steady and to risk, therefore, that will be minimal.

They compare production systems to companies, to stocks, in which investment will deliver shareholder value, will be rewarded.

As money comes into production systems, there will be liquidity for capital projects — investments in genetics, environmental programs, fencing, housing, etc., at the farm and investments in equipment and processes at the plants, including food safety and packaging processes. There will be liquidity for research and development, marketing and other uses and cash flow. Production systems will have the financing to develop their brands and market those labels around the world.

A house of protocols

How does a production system work? As mentioned earlier, it starts with the end — with consumer data that tells a coordinating unit, in the case of animal protein, what consumers are buying in beef, pork, poultry and dairy products, why and what added traits or value would get them to buy more. This information is collected by the system and analyzed by specialists systemwide (Figure, p. 1).

The system will be organized by a group of producers, management or market consultants, nutritionists or veterinarians, a feed or seed company or companies, a packer or packers, a retailer or retailers, cooperatives, private companies or, in most scenarios, combinations thereof. Indeed, it probably would be ill-advised for one company

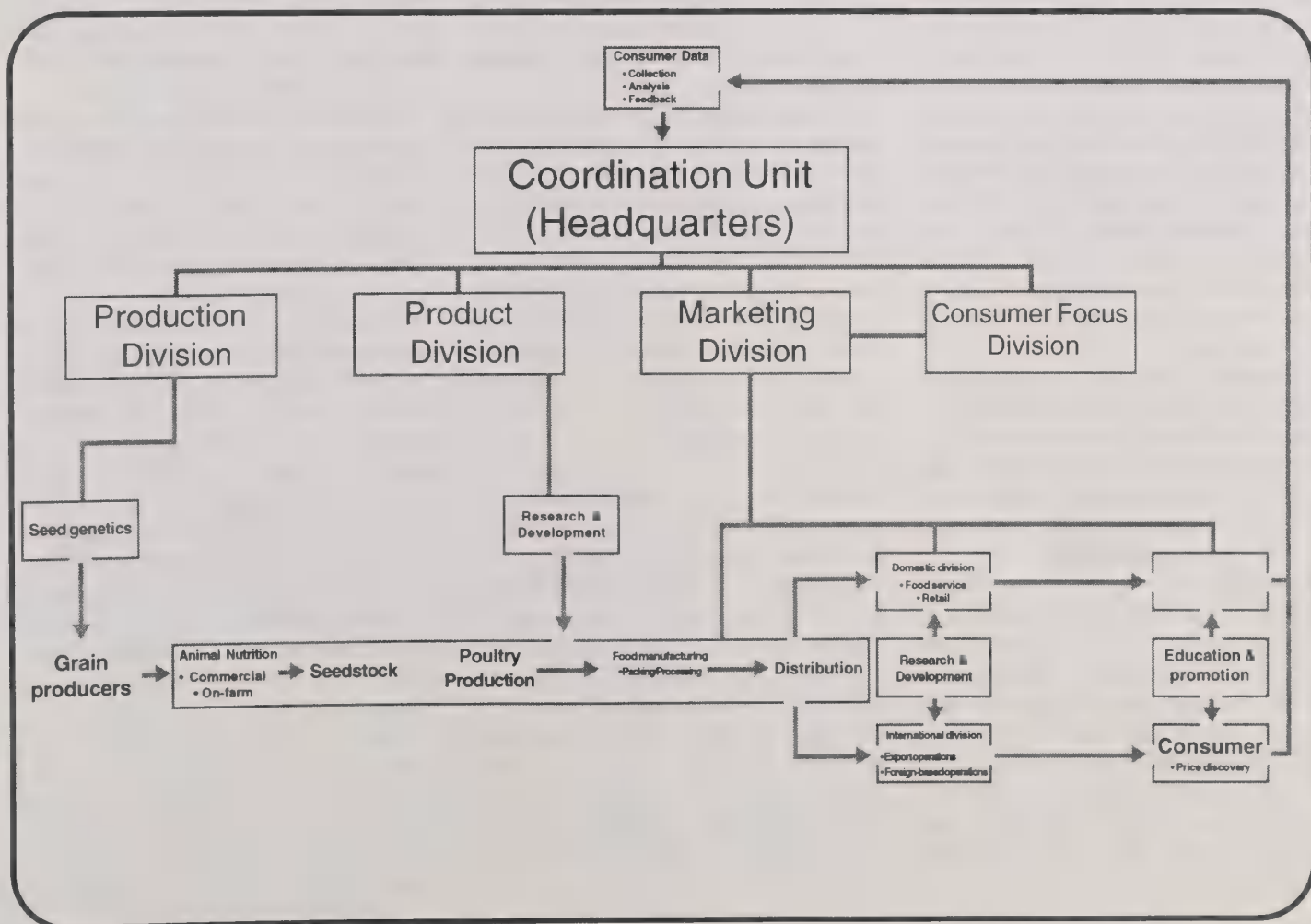
or group to try to organize a system without systemwide involvement.

The actual coordination probably, due to the size of the task, should be done by employed manager professionals.

The coordinating unit will determine how to produce, process and market the system's products, i.e., will establish production "protocols" so that the consumer products meet the consumer demand. Accordingly, the unit will determine the corn and other seed genetics needed to grow plants to process into feeds to nourish animals based on research showing those animals then will produce meat, milk or eggs that meet consumer demand.

The system then will order seeds from its seed segment for its farmer-members to grow the plants that the system needs for its next feeding and food marketing period — a food marketing period that would be out over 2-3 years in the case of beef production, or a number of food marketing periods over 12 months in the case of chicken production.

Farmers will grow only seeds authorized by the system, applying chemicals and plant foods and following production protocols found by the system as best management practices that will produce the desired quality and quantity of crops and feed inputs. Then, at



the end of the growing season, farmers will harvest and deliver those crops to the system's feed manufacturing segment.

Feed manufacturer-members then will produce feed and other nutrition products for the system's animals according to established production protocols. (Feed may be mixed commercially or on-site where feedlots, dairy and pork producers or poultry complexes have on-site operations.)

Feed and nutrition products then will be delivered to livestock and/or poultry producer-members who have signed up to produce cattle, hogs, poultry, eggs or milk for the system. As in the crop segment, producers will husband only genetics at density or stocking levels authorized by the system, following animal health and nutrition, grazing, housing, environmental, welfare and other production protocols established by the system and delivering eggs, milk or finished animals to the system's packer/processing segment.

(Established operating procedures need not be disrupted, e.g., ranchers, stockers and feedlots — and auction and other transfer points — all may participate in a beef production system if they can add competitiveness and value to the consumer product. Baby pig and feeder pig producers and finishers all may participate in a pork production system, as will farrow-to-finish producers if they can add efficiency and value to the consumer product.)

Plants will process animals and raw materials according to plant protocols, from aging to packaging, that will output branded food products. Distributors, foodservice operators, retailers and exporters who have committed themselves to the brand or brands will ship, store, handle and merchandise according to respective protocols.

Consumer education and promotion will include a consumer feedback function so that demand information will be constantly returned to the system, analyzed and acted on so that the system and its members can expand and prosper.

House and harbor

Certainly, this is an integrated model taken to a near-perfect world. However, there is nothing evil in the model, and there already are alliances and networks in the beef and pork sectors and integrators in the poultry sector (Figure, p. 18) showing that production systems are structurable and do work, and showing that the extent of perfection depends on only the accumulation of scale.

Moreover, production systems, as explained in this article, represent the way agriculture must and will go. Independent farmers and livestock and poultry producers must and will consolidate into large structures, just as have all other sectors of commerce and industry, including banks, book stores, funeral homes, hair stylists, insurance companies, petroleum companies, waste collection services and other manufacturers and retailers.

Consolidation of individual producers into production systems is necessary for individual producers to accumulate production quality and scale to compete, expand and prosper against the rigors of the marketplace in the U.S. and worldwide. Consolidation is not an option; it is indisputable and irreversible.

Yes, there will be losers. A number — perhaps a lot — of agribusinesses and producers will object to and resist merging into production systems because the process will require change — for some, probably significant change. Some will stand away from systems because they will have listened too long to voices of anger and doubt.

For those who choose to participate, though, their future will be fun. Armed with knowledge, technology and production strategies that are not only consumer-driven but demand-and-supply-oriented, they will be far more able to manage risk and realize steady returns rather than cyclical returns, including explosions in equity.

For one reason, price discovery in a production system is at the consumer level, eliminating the added costs and inefficiencies that occur when each production segment must discover prices and make a cash market, costs that decrease competitiveness. (The figure on page 1, with openings in what have always been closed production segments, suggests that production flows in a system rather than hands off in numerous ownership transfers.)

In production systems, all segments share in their system's market, and if that market is not sufficient for all segments to prosper, then the system must find ways to improve pricing and/or returns. In other words, the hamburger pays for the cow and the feed and the seed.

Actually, the point of price discovery might be better called "worth discovery." In other words, if the hamburger or pork chop or turkey drumstick or yogurt are not worth enough for all segments to prosper, then the system must find ways to make the product worth more.

Everyone is responsible for making the brand succeed — from corn seed to lunch bag to dinner table — and everyone is rewarded, with returns distributed on a basis such as a member's investment and risk in the system.

In production systems, farmers and livestock and poultry producers — from corporate producers to the largest and smallest of family farmers — are independent and have ownership interests in the success of the system. For all producers, but especially for smaller producers, it's a production system approach that will give them the efficiencies and scale they cannot accumulate individually but will need to expand, prosper and survive.

It's a production system approach that will also give producers the resources and scale — the harbor of the system — to deal with litigation and regulations related to environmental and food safety compliance, compliance that is becoming increasingly complicated and expensive and increasingly difficult for most producers to meet to stay in business, especially smaller producers.

Certainly, contracts will be necessary because there will need to be terms covering cash flows and distribution of returns, of worth. However, contracts will also be necessary to bind producers, feed companies, packers and others to conditions of membership so that all parties will be bound to conduct their business in the system according to protocols established by the system.

Contracts will be necessary to protect the integrity of the brand or brands, the members of the system and the system itself. Without a contract, a producer may sidestep or walk away from his obligations and, in doing so, damage the brand.

Subscriptions

Obviously, a lot of questions can be raised about production systems — or call them alliances, networks or supply chains. However, it has not been the purpose of this article to argue every conceivable point, to disprove or prove every conceivable point. It has not been the purpose of this article to say that American agriculture can be put in a box and everything will come out the same.

Indeed, production systems as described here are just a concept that other readers may be able to graph into a more plausible version.

This article has, rather, sought to explain that consolidation is a fact — a fact, like it or not, borne of necessity — and it has sought to show why consolidation should be joined, not resisted. It has sought to show that consolidation

may well signal something that, far from being the end of the road, is a process in which farmers and producers can participate and succeed.

It has sought to explain that a call for verticalization is not a call for integration as in the chicken industry in the Southeast, but a call for the integration of information and technology so that the diversity and independence of American agriculture can be folded into structures that will win for all those who subscribe to them.

Agriculture's leaders need to begin taking this message to the countryside, a message that the time has come to begin building brands and scale that will win in the global market, to link into systems or forms of them. Leaders must take the debate to a higher, more positive level, explain how systems will work and why they must and make commitments to putting systems together.

They need to invite those who can to come inside the house where it's warmer. ■

10/10/10
10/10/10
10/10/10
10/10/10

10/10/10
10/10/10
10/10/10
10/10/10
10/10/10
10/10/10
10/10/10
10/10/10

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

POSTER SESSION PAPERS



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Effects of Temperature on Production of Polyunsaturated Fatty Acids in Yeast Expressing a Plant Enzyme

**John M. Dyer, Dorselyn C. Chapital,
and Armand B. Pepperman
USDA-ARS-SRRC
New Orleans, LA**



Effects of Temperature on Production of Polyunsaturated Fatty Acids in Yeast Expressing a Plant Enzyme

John M. Dyer, Dorselyn C. Chapital, and Armand B. Pepperman

USDA-ARS-SRRC, 1100 Robert E. Lee Blvd, New Orleans, LA 70124

Introduction

Our interest is in developing enzyme-based technologies to convert low-cost vegetable oils into value-added products. We are currently studying the tung tree to identify enzymes involved in the synthesis of tung oil, one of the highest quality drying oils known to man. Identification of the genes and enzymes involved in tung oil biosynthesis would allow development of methods for bioconversion of low-cost vegetable oils in a tung-like drying oil.

A strategy that has been successfully used to find desaturase enzymes with a particular function, such as synthesis of tung oil, is to first identify all the desaturases in your plant based on their common sequence characteristics, and then study each enzyme independently to determine its function (1-3). Initially, the genes for the desaturase enzymes are obtained. Secondly, the genes are expressed in a different organism that does not normally make your fatty acid of interest and finally look for the appearance of that fatty acid.

Several groups have successfully expressed plant fatty acid desaturase genes in the common baker's yeast *Saccharomyces cerevisiae* (4-6). This is an excellent model system since it is very easily to manipulate genetically, and the yeast do not contain polyunsaturated fatty acids (PUFA). This makes it very easy to study the function of plant desaturase genes that you suspect might be involved in synthesis of polyunsaturated fatty acids. Even if the enzyme produces very small amounts of product, such as linoleic acid (C18:2), the appearance of the fatty acid will be easy to determine since it is completely absent in normal yeast lipids.

The amount of fatty acid produced by plant desaturases in yeast can vary quite dramatically. In some cases the product of the enzyme can account for about 15% of the yeast fatty acid content, but more often it accounts for less than 1%. In some instances, a greater amount of the desired fatty acid can be synthesized when the yeast are grown at cooler temperatures (4). In many organisms, including plants, growth at lower temperatures induces accumulation of higher amounts of PUFA. It is thought that the increase in PUFA helps maintain a fluid-like state of lipid membranes at low temperatures. However, common baker's yeast did not evolve in the presence of PUFA, and therefore probably has some other mechanism to protect membranes. Understanding these temperature effects could illuminate avenues of research that could substantially improve the activity of plant desaturases expressed in yeast.

Since we are interested in studying plant enzymes involved in synthesis of trienoic fatty acids, we established a positive control system in yeast by expressing the rapeseed gene involved in synthesis of linolenic acid (C18:3). This gene (FAD3) produces an enzyme that converts linoleic

acid (C18:2) into linolenic acid (7). Experiments are also presented that determine how temperature affects the amount of product produced by the plant enzyme in yeast.

Materials and Methods

Yeast culture conditions. Yeast were cultured in liquid media containing 2% galactose, 0.67% yeast nitrogen base, and appropriate amino acid supplements. In some experiments, fatty acids were included in the media at 0.1% v/v final. Cells were incubated at 30 (normal growth temperature), 20, or 10 °C with shaking at 300 RPM. All cultures were grown to near saturation then harvested by centrifugation.

Lipid extraction and analysis. Yeast cells were converted to spheroplasts by enzymatic degradation of the cell walls (8). Total lipids were extracted from spheroplasts using the methanol/chloroform procedure of Bligh and Dyer (9). Fatty acid methyl esters were prepared using methanolic-HCl, and FAME were analyzed using GC. All experiments were performed at least three times and statistical significance was determined using ANOVA.

Expression of rapeseed FAD3 gene in yeast. The FAD3 gene was obtained from the Arabidopsis Biological Resource Center (<http://aims.cps.msu.edu/aims>) and cloned into a yeast expression vector. A second version of FAD3 was generated that included a short peptide sequence to serve as a “tag” to monitor levels of the FAD3 enzyme in the cells. The two FAD3 genes were inserted into separate yeast strains. Yeast were grown and lipids extracted and analyzed as above.

Analysis of FAD3 enzyme levels in yeast. Total protein was isolated from yeast cells expressing the tagged version of FAD3. Proteins were separated on two identically prepared SDS-polyacrylamide gels. One gel was stained with Coomassie blue, which stains all proteins. Proteins in the second gel were transferred to nylon membrane and the FAD3 protein was detected using an antibody that specifically recognized the “tag”. Experiments were performed at least twice and a representative figure is shown.

Results

Uptake of fatty acids into common baker's yeast. Before attempting to express the rapeseed FAD3 gene in yeast for synthesis of linolenic acid, we evaluated whether the substrate or product of the enzyme might be toxic to yeast. This is important since yeast do not normally contain either linoleic or linolenic acid. As shown in Figure 1, the fatty acid composition of normal yeast is dominated by four fatty acids: palmitic (9%), palmitoleic (15%), stearic (15%), and oleic (60%). Growth of yeast on media containing linoleic and linolenic acids led to substantial incorporation into yeast lipids, accounting for 61% of yeast fatty acids. Although fed in equal proportions in the media, linolenic acid accounted for a larger percentage (44%) of fatty acid composition than linoleic acid (17%). Uptake of linoleic and linolenic acids led to a substantial decrease in the amount of the normal yeast unsaturated fatty acids, palmitoleic and oleic. This phenomenon was observed previously with yeast fed oleic or linoleic acid (10). These results demonstrate that yeast can tolerate substantial amounts of polyunsaturated fatty acids.

Synthesis of linolenic acid in yeast expressing the rapeseed FAD3 gene. Yeast cells containing the rapeseed FAD3 gene were grown on media supplemented with linoleic acid, the substrate of the FAD3 enzyme. Extraction and analysis of lipid components revealed the presence of small amounts of linolenic acid (Figure 2), verifying that the FAD3 enzyme was capable of synthesizing linolenic acid. Linolenic acid was not detected in negative control cells that lacked the FAD3 enzyme. Growth of yeast at cooler temperatures led to an increase in the amount of linolenic acid produced. The order of temperatures, from condition of highest amount of linolenic acid to lowest, was 20°C > 10°C > 30°C.

Effects of chilling temperature on the lipid composition of yeast. To determine if the increase in linolenic acid at lower temperatures might be explained by an increased need for PUFA at lower temperatures, normal yeast cells (lacking the FAD3 gene) were fed a combination of linoleic and linolenic acid and cells were grown at 30, 20, and 10 °C. As shown in Figure 3, yeast cells do not accumulate more PUFA at lower temperature. The amounts of linoleic and linolenic acid actually decreased at lower temperatures. Interestingly, the amount of short chain fatty acids (C16) increased at cooler temperatures. This might afford a similar protective effect, since short chain fatty acids remain fluid at lower temperatures much better than their longer chain counterparts. Thus, there is no correlation between the amount of linolenic acid synthesized at various temperatures by FAD3 and an increased cellular demand for PUFA at lower temperatures.

Effects of chilling temperature on levels of FAD3 enzyme in yeast. To determine if temperature might have some direct effect on levels of the FAD3 enzyme in yeast cells, proteins were extracted from cells grown at the various temperatures and equal amounts of protein from each sample were separated on an SDS-gel. The levels of FAD3 were determined using an antibody specific for the enzyme, as described in Materials and Methods. As shown in Figure 4, there was very little FAD3 detected in cells grown at 30 °C, while there was significantly more at 20 °C, and slightly less at 10 °C. This pattern in amount of desaturase enzyme, (20°C > 10°C > 30°C), matches the pattern of linolenic acid produced by FAD3 at the various temperatures.

Conclusions

The rapeseed FAD3 gene was successfully expressed in yeast and resulted in synthesis of linolenic acid. This suggests that yeast can serve as a useful model system to characterize plant genes involved in trienoic fatty acid biosynthesis. The production of linolenic acid by rapeseed FAD3 was very low (< 1%), but easily detectable over negative controls, demonstrating the sensitivity of this assay. Production of linolenic acid was sensitive to temperature, with highest amounts observed at 20°C > 10°C > 30°C. The increased amount of linolenic acid at cooler temperatures was not due to an increased cellular demand for polyunsaturated fatty acids. Rather, lower temperatures had a direct effect on the amount of FAD3 enzyme detected in the cells. The highest amounts of FAD3 enzyme were detected at 20°C > 10°C > 30°C, which directly correlates with the amount of linolenic acid detected at the various temperatures (summarized in Figure 5). Current experiments focus on methods to improve enzyme stability and explore other modifications of this system for possible industrial production of exotic fatty acids using plant enzymes.

References

1. van de Loo, F. J., Broun, P., Turner, S., and Somerville, C. (1995). An oleate 12-hydroxylase from *Ricinus communis* L. is a fatty acyl desaturase homolog. *Proc. Natl. Acad. Sci. U. S. A.* 92, 6743-6747.
2. Lee, M., Lenman, M., Banas, A., Bafor, M., Singh, S., Schweizer, M., Nilsson, R., Liljenberg, C., Dahlqvist, A., Gummesson, P. O., Sjodahl, S., Green, A., and Stymne, S. (1998). Identification of non-heme diiron proteins that catalyze triple bond and epoxy group formation. *Science* 280, 915-918.
3. Cahoon, E. B., Carlson, T. J., Ripp, K. G., Schweiger, B. J., Cook, G. A., Hall, S. E., and Kinney, A. J. (1999). Biosynthetic origin of conjugated double bonds: Production of fatty acid components of high-value drying oils in transgenic soybean embryos. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12935-12940.
4. Covello, P. S., and Reed, D. W. (1996). Functional expression of the extraplastidial *Arabidopsis thaliana* oleate desaturase gene (*FAD2*) in *Saccharomyces cerevisiae*. *Plant Physiol.* 111, 223-226.
5. Kajiwar, S., Shirai, A., Fujii, T., Toguri, T., Nakamura, K., and Ohtaguchi, K. (1996). Polyunsaturated fatty acid biosynthesis in *Saccharomyces cerevisiae*: Expression of ethanol tolerance and the *FAD2* gene from *Arabidopsis thaliana*. *Appl. Environ. Microbiol.* 62, 4309-4313.
6. Brown, A. P., Dann, R., Bowra, S., and Hills, M. J. (1998). Characterization of expression of a plant oleate desaturase in yeast. *J. Am. Oil Chem. Soc.* 75, 77-82.
7. Arondel, V., Lemieux, B., Hwang, I., Gibson, S., Goodman, H. M., and Somerville, C. R. (1992). Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. *Science* 258, 1353-1355.
8. Dyer, J. M., McNew, J. A., and Goodman, J. M. (1996). The sorting sequence of the peroxisomal integral membrane protein PMP47 is contained within a short hydrophilic loop. *J. Cell Biol.* 133, 269-280.
9. Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911-917.
10. Bossie, M. A., and Martin, C. E. (1989). Nutritional regulation of yeast Δ -9 fatty acid desaturase activity. *J. Bacteriol.* 171, 6409-6413.

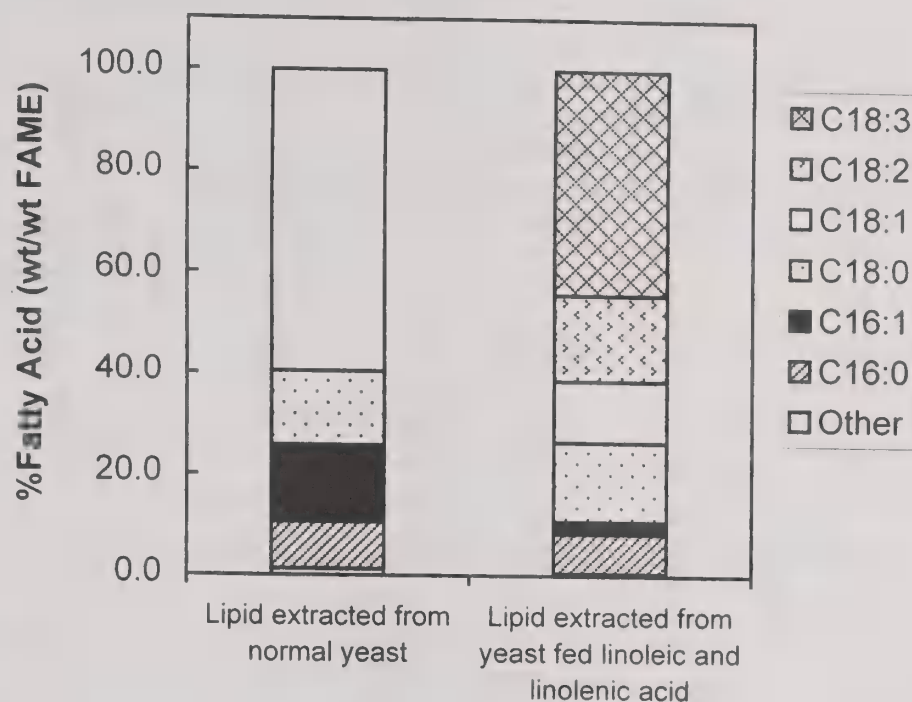


Figure 1. Uptake of linoleic and linolenic acid into yeast.

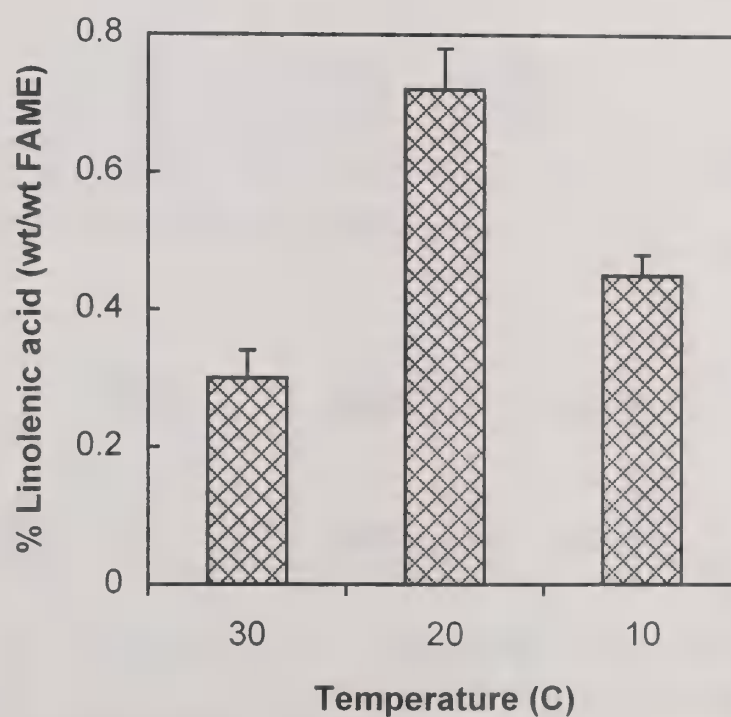


Figure 2. Synthesis of linolenic acid in yeast expressing the rapeseed FAD3 gene. Yeast were cultured at the indicated temperatures, lipids extracted, and FAME analyzed by GC. Linolenic acid was not detected in negative control cells lacking the FAD3 gene.

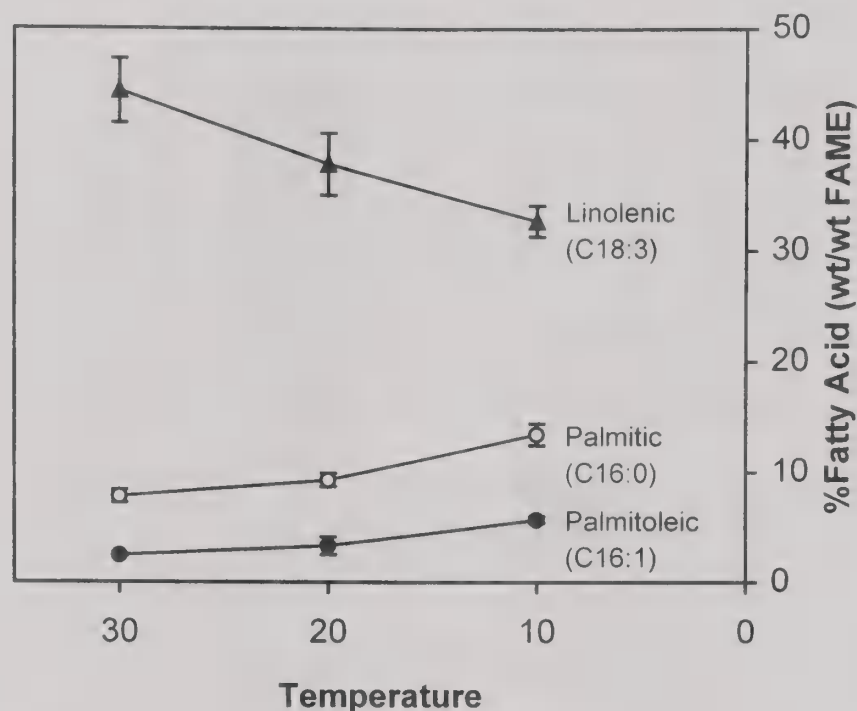


Figure 3. Changes in fatty acid composition of yeast during chilling. Yeast cultures were supplemented with linoleic and linolenic acid and cells were grown at the indicated temperatures. Lipids were extracted and FAME analyzed by GC. Other fatty acids showed little change in percent composition.

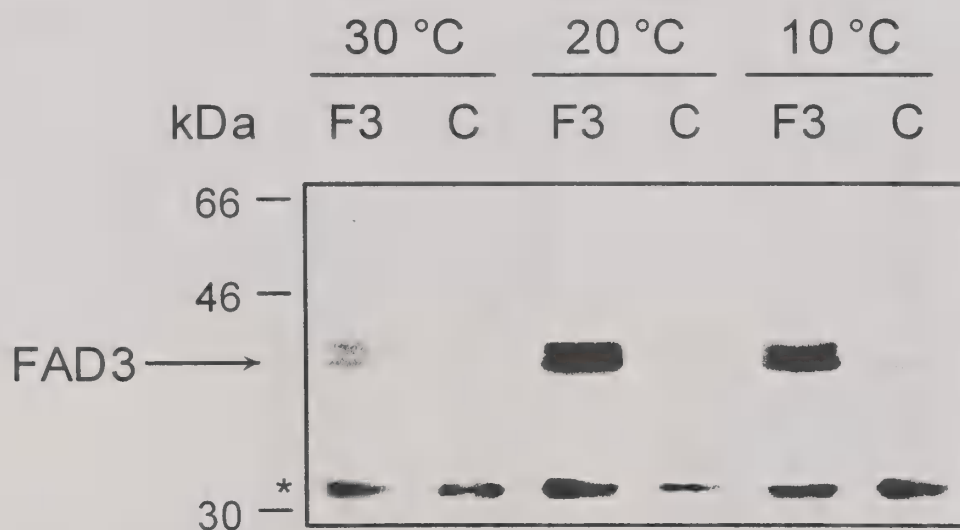


Figure 4. Level of FAD3 enzyme in yeast cells. Yeast containing the FAD3 gene were grown at the indicated temperature then total protein was extracted. An equal amount of protein was separated on an SDS-polyacrylamide gel, transferred to nitrocellulose, and developed using an antibody specific to FAD3 (arrow). The positions of molecular mass markers are shown at the left. F3, cells expressing FAD3 gene; C, control cells lacking FAD3; *, a cross-reacting band present in all cells that serves as a control to show equal loading of protein.

Temperature (°C)	Amount of long chain unsaturated fatty acids in yeast	Amount of linolenic acid synthesized by rapeseed FAD3 in yeast	Amount of FAD3 in yeast cells
30	+++	+	+
20	++	+++	+++
10	+	++	++

Figure 5. Summary of data. In normal yeast cells, there was a progressive decrease in the amount of long chain unsaturated fatty acids as the cells were chilled. Despite the reduction in cellular requirement for unsaturated fatty acids, a greater amount of linolenic acid was synthesized by the FAD3 enzyme in yeast grown at cooler temperatures. This increase could be attributed to the increase in amount of FAD3 levels in the cells. The FAD3 enzyme is likely to be unstable in yeast, and therefore cooler temperatures may help to stabilize the enzyme structure.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Formulation, Structure and Properties of Commercial Spreads, a 1999 Survey

G.R. List, K.R. Steidley, and W.E. Neff
Agricultural Research Service, USDA
Peoria, IL



49th Oilseeds Conference, New Orleans, LA, March 19-21, 2000

Formulation, Structure and Properties of Commercial Spreads, a 1999 Survey

G.R. List, K.R. Steidley and W.E. Neff

Abstract

Consumer demands for more healthy food products over the past several decades have resulted in margarines and spreads which have been lowered in fat, saturated fat, *trans* fat and total calories. Technological advances in product preparation have allowed such changes in formulation without sacrificing functionality. Continued improvement in nutritional and functional properties and compliance with proposed *trans* fat labeling regulations will be the focus of the margarine/spread industry for at least the near future. Historically, formulation of margarine/spread oils have been primarily guided by the solid fat index/content and melting point of the hydrogenated components. Relatively little has been reported on the structures of triglycerides in hydrogenated fats. High performance liquid chromatography (HPLC) was investigated for the separation and quantitation of triglycerides of unhydrogenated liquid oils and margarine/spread base stocks. Although the method will not resolve triglyceride positional isomers and *trans* isomers affect resolution, much information can be gained including melting points of individual triglycerides, determination of iodine values, triglyceride structures and quality control. Applications of the HPLC method to these problems will be presented and the results discussed. Data are presented showing that the average *trans* acid content of soft tub spreads has been reduced by 55% over the period 1992-1999 and the *trans* content of stick products reflect a 37% reduction over a 10 year period. Reductions in *trans* acids can be attributed to shifts from a multiple hydrogenated basestock system, use of more liquid oils and, to a lesser extent, alternative oil processing techniques such as interesterification.

Introduction

Over the past several decades, the margarine industry has undergone much change (1-6). Driven by consumer demands for fewer calories, standard 80% fat margarines have been largely replaced by so called spreads containing 40-70% fat. Other factors impacting the industry stem from health and nutritional issues leading to the use of more liquid oils, thereby increasing levels of polyunsaturated fatty acids. *Trans* acids resulting from hydrogenation will likely impact the industry as a result of labeling regulations recently proposed by the Food and Drug Administration (7). Historically, margarine, spread and shortening oils have been formulated from a base stock system employing a number of oils hydrogenated to standard iodine values, melting points and solid fat index properties (8,9). The properties of these oils within a typical hydrogenated soybean oil base stock system have been described by Latondress (9) who points out that by blending these products, either with each other or in combination with liquid oil, permits the formulation of a wide variety of margarines/spread oils. The primary quality control tools used by the industry employ solid fat index (SFI) or solid fat content (SFC) determined by dilatometry and NMR respectively.

The structures of hydrogenated triglycerides have received little attention and, owing to the complexity of these products, fundamental questions including specificity for hydrogenation of the 1 or 2 position of triglycerides, as well as triglyceride selectivity remain controversial topics (10).

Names are necessary to report factually on available data; the USDA neither guarantees nor warrants the standard of the product, and the use of the name USDA implies no approval of the product to the exclusion of others that may also be suitable.

Experimental

Margarines and spreads were obtained from local supermarkets. The products were transferred to glass beakers, placed in a microwave oven and, after melting the oil, were recovered by centrifugation. Fatty acid compositions were determined by capillary gas chromatography after transesterification. Triglycerides were separated by high pressure liquid chromatography as described previously (11,12). Dropping points and solid fat index determinations were carried out according to official AOCS methods (13).

High Performance Liquid Chromatography

Reversed phase (RP)-HPLC was performed with a Thermo Separation Products (Schaumburg, IL) (Model SP 8800) ternary solvent system with two RP-HPLC columns with bonded silyl (CT8) ODS, Inertsil ODS-80A, GL Sciences, Keystone Scientific (Bellfonte Park, PA). 25 cm x 4.6 mm, 5 μ m in series. The gradient elution was as follows: 80% acetonitrile (ACN), 20% dichloromethane (DCM) to 20% ACN, 80% DCM after 120 minutes. The flow rate was 0.6 ml/min. throughout. Sample size (25 μ g) injected was 10 μ l of 25 mg solute/ml DCM.

Evaporative Light Scattering Detector

The ELSD was a Sedex Model 55, Sedone (Altonville, France). The drift tube was set at 32°C. The gas flow was set at a pressure of 1.6 bar. The photo multiplier gain was times five. High purity N₂ was used as the nebulizer gas.

Triacylglycerol Identification

The triacylglycerol HPLC chromatogram peaks were identified based on earlier analysis of SBO via reversed phase HPLC coupled with an atmospheric pressure chemical ionization mass spectrometer (14,15).

Data Processing

The data output from ELSD was processed or integrated by a Star Chromatography Workstation with version 4.0 software, Varian Associates, Inc. (Walnut Creek, CA).

Gas Chromatography

Fatty acid methyl esters (FAME) were prepared by the potassium hydroxide catalyzed transmethylation of the TAG mixtures and the FAME were analyzed using calibrated gas chromatography (GC) (16).

Results and Discussion

The fatty acid composition and physical properties of seventeen commercial margarines and spreads are given in Table 1. Included are data for 9 soft tub and 8 stick products. Solid fat index (SFI) and drop melting points are in agreement with published values (17). Excellent agreement was obtained between iodine values obtained by HPLC of the triglycerides and those calculated from gas chromatography of the methyl esters. *Trans* acid content of the soft tub products ranged from 2.6 to 14.6%, while the stick products ranged from 3.2 to 25.8%. A survey of the composition and properties of a number of soft tub margarines published in 1991 indicated that products formulated from hydrogenated soybean oil in the United States contained *trans* acids levels ranging from 10.3-12.4% (17). The *trans* content of Canadian margarines ranged from 12.6-14.1% (17).

According to their labels, most of these products were formulated from either hydrogenated soybean oil or from blends of hydrogenated and liquid soybean oil. Although the fatty acid compositional data are in agreement with this approach, the individual triglyceride profile provide additional information. The HPLC triglyceride profiles of a number of common unhydrogenated vegetable oils used in margarine/spread formulations are shown in Table 2.

TABLE 1. Composition and physical properties of commercial margarine/spread oils.

Sample	% Fat	Oil Components ^a	Solid Fat Index (°C)					Drop Melting Point (°C)	Iodine Value		% Trans Acids	Fatty Acids by FAME-GC					
			10	21.1	26.7	33.3	40		TG-HPLC	FAME- GC		14:0	16:0	18:0	18:1	18:2	18:3
Tub																	
A	68	PHSO	10.4	5.8	4.0	2.1	0.3	32.7	101.2	104.3	14.6	0.0	10.8	6.1	47.0	34.5	1.6
B	80	L, PHSO	9.3	4.9	3.8	1.9	0.7	32.2	112.8	114.2	7.9	0.0	11.9	5.9	36.7	41.1	4.4
C	60	L, PHSO	8.3	5.4	4.3	2.5	1.4	31.2	118.3	119.1	5.3	0.1	11.5	7.3	30.8	44.3	6.1
D	70	L, PHSO	9.0	5.3	4.1	2.2	0.4	31.8	119.4	120.0	6.1	0.1	10.7	7.2	31.7	44.1	6.3
E	70	PHSO	11.5	6.6	3.7	1.7	0.1	32.6	98.0	99.9	14.5	0.1	12.6	6.5	47.3	32.0	1.5
F	48	L, PHSO	9.0	5.6	4.2	2.2	0.8	32.2	119.9	119.5	5.4	0.0	11.7	7.0	30.6	44.7	6.1
G	60	LSO,HSO	10.1	4.8	3.0	1.6	0.3	33.1	118.6	118.3	2.6	0.0	7.3	12.1	25.6	53.9	1.1
		PHSO,LCO															
H	80	L, PHSO	13.9	8.6	4.7	0.7	0.6	31.5	110.7	115.3	10.5	0.0	10.5	6.4	38.0	39.5	5.6
I	60	L, PHSO	13.7	7.5	4.5	1.3	0.2	31.6	112.2	113.7	10.5	0.3	11.1	6.7	38.1	38.3	5.6
Stick																	
J	80	L, PHSO	24.8	13.0	7.7	1.7	0.1	33.0	79.5	82.9	25.8	0.2	13.5	6.1	64.8	15.1	0.4
K	70	L, PHSO	20.4	10.7	7.5	3.1	0.2	33.8	96.1	94.9	18.2	0.0	13.1	6.5	52.9	25.7	1.9
L	40	L, PHSO	19.7	10.3	5.1	0.3	0.2	31.8	94.7	96.7	19.0	0.0	12.3	5.7	53.9	26.4	1.8
M	68	LSO,HSO	13.7	7.4	4.7	2.5	0.4	35.1	101.7	105.6	3.2	0.0	7.3	17.9	29.0	44.2	1.5
		PHSO,LCO															
N	60	L, PHSO	19.3	9.0	5.6	1.7	0.1	32.1	96.8	97.8	16.7	0.0	13.2	6.1	51.2	26.4	3.1
O	70	L, PHCO	20.3	12.3	8.4	3.2	0.3	34.0	103.3	104.6	14.8	0.0	12.0	5.9	43.8	37.8	0.6
P	53	L, PHSO	21.6	12.0	7.9	2.5	0.3	32.9	91.8	95.7	18.2	0.0	11.5	8.1	51.9	26.7	1.9
Q	40	L, PHSO	22.4	12.4	8.8	3.0	0.2	33.7	90.4	95.0	19.7	0.0	10.8	7.8	54.9	24.6	2.0

^a PHSO = Partially hydrogenated soybean oil

L, PHSO = Liquid and partially hydrogenated soybean oil

LSO = Liquid sunflower oil

HSO = Hydrogenated soybean oil

LCO = Liquid canola oil

L, PHCO = Liquid and partially hydrogenated corn oil

TABLE 2. Triacylglycerides of common vegetable oils by high pressure liquid chromatography-flame ionization detection

TAG ^a	Canola oil	Peanut oil	Corn oil	Cottonseed oil	Soybean oil	Mid-oleic Sunflower oil	Regular Sunflower oil
LnLnLn	0.1	0.0	0.0	0.0	0.0	0.0	0.0
LnLnL	0.3	0.0	0.0	0.1	0.7	0.0	0.0
LnLL	1.2	0.1	0.6	0.1	6.3	0.1	0.0
LnLnO	1.3	0.0	0.0	0.1	0.2	0.0	0.0
LnLnP	0.1	0.0	0.0	0.0	0.1	0.0	0.0
LLL	2.7	1.9	23.4	18.8	17.2	11.5	32.4
LnLO	6.4	0.2	0.9	1.2	5.0	1.0	1.1
LnLP	1.0	0.1	0.4	0.8	2.5	0.1	0.2
LLO	9.0	13.0	23.4	12.6	17.9	12.1	27.9
LnOO	9.6	0.4	0.5	0.5	1.3	0.9	0.4
LLP	3.0	3.8	15.7	27.8	12.9	4.1	10.7
LnOP	1.8	0.2	0.4	1.6	1.1	0.2	0.3
LnPP	0.1	0.6	0.3	0.3	0.1	0.0	0.2
LOO	21.7	20.1	10.9	3.7	9.5	8.3	6.7
LLS	1.0	0.9	2.1	1.8	3.2	2.7	7.4
LOP	5.8	11.8	10.4	12.9	9.2	2.6	4.8
PLP	1.1	2.8	1.7	10.8	1.6	0.1	0.7
OOO	23.6	15.7	3.3	1.0	3.1	40.2	1.7
LOS	1.3	2.4	1.5	0.8	2.7	1.6	2.2
POO	4.8	8.7	2.4	0.0	2.3	5.7	0.6
SLP	1.1	4.8	0.6	1.4	0.9	0.4	0.7
POP	0.3	1.8	0.6	1.3	0.6	0.8	0.8
PPP	0.3	1.0	0.2	1.9	0.1	0.1	0.1
SOO	1.4	3.0	0.4	0.1	0.7	5.4	0.4
SLS	0.3	1.6	0.2	0.2	0.2	0.3	0.3
SOP	0.4	1.4	0.2	0.2	0.3	0.3	0.3
PPS	0.1	2.0	0.1	0.1	0.1	0.1	0.1
SOS	0.1	0.7	0.1	0.0	0.0	0.9	0.1
PSS	0.0	0.7	0.0	0.0	0.0	0.1	0.1
SSS	0.0	0.2	0.0	0.0	0.0	0.2	0.0

^aLn=Linolenic, L=Linoleic, O=Oleic, P=Palmitic, S=Stearic

Corn, cottonseed, soybean and sunflower oil all contain 3 triglycerides that serve as markers to estimate the proportions of unhydrogenated oil used in margarine/spread oils. These include

trilinolein (LLL), dilinoleyl-olein (LLO) and dilinoleyl-palmitin (LLP). Since high pressure liquid chromatography will not resolve positional isomers, the LLO peak contains LOL as well. Since palmitic and stearic acid are essentially absent from the 2 position of vegetable oil triglycerides, the LLP peak contains very little LPL. Canola and mid oleic sunflower triglycerides contain high levels of triolein and dioleyl-linolein which can be used as markers in margarine/spread oils. However, since triolein is a major product resulting from hydrogenation, its presence may complicate interpretation of the HPLC data, as will be discussed later.

The effects of hydrogenation on the triglyceride structure of vegetable oils has received little attention. We recently reported the effects of pressure on the selectivity of hydrogenation of soybean oil at pressures of 50-500 lbs H₂, a temperature of 120°C and vigorous stirring in a batch reactor. (18) At high pressures, where a hydrogen rich environment exists on the catalyst surface, the linoleate containing triglycerides LLL, LLO and LLP are strongly adsorbed favoring hydrogenation. At lower pressures other reactions take place, including isomerization from *cis* to *trans*, desorption from the catalyst surface and readsorption, where the cycle may be repeated. At high pressures (500 lbs H₂) hydrogenation is truly non-selective, since di and trisaturates are formed at iodine values of about 70. *Trans* acid levels are relatively low. At lower pressures (50 lbs H₂) the reaction becomes more selective since di and trisaturated triglycerides are essentially absent while the *trans* acids levels become elevated (22%).

Tub product G and stick product M (Table 1) are low *trans* products containing 2.6 and 3.2% *trans* respectively and appear to be formulated by both interesterification and hydrogenation. The elevated stearic acid contents suggest that interesterified base stock(s) high in stearic acid were blended with liquid oils to achieve the desired solid fat index profiles. Of the 9 tub products shown in Table 1, only 2 (A and E) are formulated from all hydrogenated oils while 7 are formulated from a single hydrogenated base stock and liquid oil. Oils high in LLL, LLO and LLP are indicative of unhydrogenated oils in their formulations.

Stick products contain higher amounts of triolein (OOO) which are undoubtedly complex mixtures of both *cis* and *trans* isomers. Common liquid vegetable oils high in triolein include canola, peanut and mid-oleic sunflower oil. However, soybean and corn are low in triolein which increase upon hydrogenation. Thus high levels of triolein in a margarine/spread oil may result from either the liquid oil or the hydrogenated component.

Stick and spreadable stick products require higher solid fat index profiles than soft tub spreads which limit the amount of liquid oil which can be incorporated into the formulation. For example, a typical tub margarine oil formulated from 25-30% and 70-75% hydrogenated soybean oil (I.V. 65) will contain about 10% *trans* acids and have solid fat index values of 12 @ 10°C, 4-5 @ 21.1°C and 2 @ 33.3°C. To achieve SFI values suitable for stick and spreadable stick the ratio of basestock to liquid oil must be increased to about 50:50, thereby increasing the *trans* acids levels to about 20%. The data shown in Table 3 suggests that, while some liquid oils are incorporated into stick products, most are formulated from a multiple basestock system employing 2 or more hydrogenated oils.

Triglyceride data from the stick margarines/spreads are more difficult to interpret because hydrogenation decreases the amounts of LLL, LLO and LLP which, in turn, is dependent on the conditions employed during hydrogenation. For example, the triglyceride profile of commercially prepared hydrogenated-winterized soybean oil (I.V. 107) showed LLL, LLO and LLP contents of 4.2, 10, and 6.1%. With the exception of stick product M, formulated largely by interesterification, all other products are consistent with formulation from largely hydrogenated oils as opposed to higher amounts of liquid oils used in tub products. Stick products are characterized by elevated levels of

OOO, OOP, OOS and SSO, all of which are products resulting from the hydrogenation of LLL, LLO and LLP.

The linolenic acid content of soybean oil usually ranges 7-8%. Thus, based on the data given in Table 1, soft tub products formulated with liquid and partially hydrogenated oils contain approximately 50-70% unhydrogenated oil, whereas the stick products contain about 20-30%. The trilinolein (LLL) content of unhydrogenated soybean oils we have examined varied from 15-19%. A heavily hydrogenated (IV 65-70) soybean oil basestock suitable for blending with liquid soybean oil to formulate either soft or stick margarines contains no trilinolein and, on this basis, the stick products shown in Table 1, with the exception of M and O, contain about the same amount of unhydrogenated oil as indicated from their linolenic acid contents. Samples K, L, P and Q contain about 5% trilinolein, which amounts to about 30% of the amount found in unhydrogenated soybean oil (Table 2).

TABLE 3. Triglycerides of Margarines/Spread Oils by HPLC.

TAG	Tub Products									Stick Products							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
LnLnLn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
LnLnL	0.0	0.4	0.8	0.7	0.0	0.8	0.2	0.6	0.7	0.0	2.0	0.2	0.2	0.3	0.1	0.0	0.0
LnLL	0.5	3.4	5.5	5.6	0.5	5.8	0.3	4.8	5.6	0.2	1.3	1.1	0.2	2.7	0.5	1.1	1.4
LnLnO	0.5	1.0	0.2	0.7	0.4	0.5	0.0	0.7	0.1	0.1	0.3	0.4	0.1	0.3	0.1	0.5	0.3
LnLnP	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.2	0.0	0.1	0.0	0.2	0.1	0.1	0.0	0.0
LLL	3.6	12.6	14.3	14.5	3.8	14.8	26.3	12.1	12.4	1.0	5.1	4.8	17.9	7.5	13.5	5.1	4.8
LnLO	3.6	3.6	4.2	4.4	3.1	4.3	0.1	4.0	4.3	0.7	2.2	2.3	1.7	2.2	1.1	2.5	1.8
LnLP	0.7	1.7	2.3	2.5	0.6	2.5	0.4	2.2	2.2	0.2	0.8	0.8	0.4	1.2	0.3	0.9	0.8
LLO	9.3	13.4	14.3	13.9	10.3	13.6	21.0	12.0	12.1	2.4	7.1	6.7	15.9	7.5	14.0	7.5	6.3
LnOO	5.1	0.9	0.6	0.7	3.8	1.0	0.5	0.8	0.9	1.6	1.5	2.0	1.1	0.6	0.9	1.7	1.4
LLP	6.5	10.2	11.3	11.2	5.5	10.8	8.5	8.9	9.1	1.9	5.0	4.8	6.4	5.7	8.6	5.0	4.2
LnOP	2.1	0.6	0.7	0.5	1.3	1.0	0.6	0.9	0.8	0.5	0.8	0.9	0.7	0.5	0.9	0.9	0.6
LnPP	0.1	0.1	0.0	0.2	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0
LOO	12.2	6.9	6.5	5.8	11.5	6.1	5.3	5.4	5.4	7.4	5.8	5.8	5.0	4.4	7.0	6.0	4.8
LLS	6.1	3.0	3.2	3.2	6.9	3.1	5.9	2.6	2.7	2.5	3.7	3.8	4.9	2.8	1.6	3.7	4.8
LOP	13.6	8.3	8.0	7.6	11.5	7.5	5.0	6.6	6.7	10.8	7.3	8.1	5.1	5.9	7.2	7.5	6.9
PLP	1.6	1.7	1.7	1.7	1.5	1.7	0.7	1.5	1.5	0.1	1.1	1.3	0.8	1.5	1.3	1.4	1.1
OOO	5.4	4.3	2.8	3.2	5.5	4.0	2.4	3.6	2.6	23.3	21.2	30.7	2.9	23.3	9.9	18.0	23.4
LOS	8.5	7.1	5.9	5.7	7.7	5.6	4.8	8.0	9.7	4.7	14.1	3.9	5.9	8.3	12.8	15.2	12.5
POO	7.7	8.2	4.2	4.4	11.1	4.5	1.8	7.3	5.2	26.9	7.0	8.0	2.2	10.4	4.4	3.1	7.6
SLP	4.9	4.6	3.1	3.7	4.5	3.1	2.4	6.1	7.7	2.5	3.1	4.6	3.7	2.3	4.1	3.7	2.6
POP	1.6	2.2	1.3	1.3	1.8	0.8	1.2	1.0	1.4	1.4	1.4	0.0	1.1	2.0	0.7	2.1	0.9
PPP	0.1	0.0	0.2	0.3	0.8	0.2	0.1	0.9	0.4	0.0	2.5	3.4	0.2	0.6	0.0	0.8	0.8
SOO	2.2	3.0	3.3	1.9	2.1	2.0	2.8	1.7	1.7	6.5	4.7	3.6	5.0	5.2	1.8	4.4	6.1
SLS	1.8	0.5	1.4	3.1	2.7	2.7	2.3	4.3	3.8	1.0	1.5	1.3	4.1	1.7	5.6	4.4	2.1
SOP	1.6	1.5	2.1	2.1	2.4	1.9	2.3	2.2	2.0	3.5	1.1	0.9	3.8	2.2	2.0	3.5	3.5
PPS	0.0	0.1	0.2	0.1	0.2	1.0	4.3	0.2	0.5	0.1	0.2	0.4	9.3	0.2	0.9	0.8	0.2
SOS	0.4	0.2	1.2	0.7	0.5	0.1	0.3	0.8	0.1	0.2	0.4	0.1	0.8	0.3	0.1	0.1	0.7
PSS	0.1	0.1	0.2	0.1	0.1	0.0	0.0	0.2	0.0	0.2	0.3	0.0	0.2	0.2	0.0	0.0	0.2
SSS	0.1	0.3	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.2	0.3	0.1	0.3	0.1	0.1	0.1	0.1

The *trans* content of seven soft margarines/spreads taken over the years 1992-1999 are shown in Table 4. The data clearly shows that the industry has made a concerted effort to lower the *trans* acid levels of margarine/spread products. The average *trans* content in 1992 was 19.5% which dropped to 16.1% by 1995 and the present study shows an average of 8.8% amounting to a 55.8% total reduction in *trans* acids over the 7 year period.

A survey of the composition and physical properties of North American stick margarines, published in 1989, showed that Canola based margarine produced in Canada contained 33.1-45% *trans* (19), whereas, soybean oil based margarine contained 22.4-30.1% *trans* (average 26.8%). Data for American stick products (Table 1) show an average of 16.9% *trans* which includes the 3.2% sample.

The other 7 samples average 18.8% *trans*. Thus, over the past decade, the *trans* content of American stick margarines have been reduced by 37%.

TABLE 4. *Trans* contents of soft margarines/spreads by year.

Brand	Year			Overall Reduction %
	1992	1995	1999	
1	19.4	15.9	14.5	25.3
2	10.3	18.2	7.9	23.4
3	12.2	7.8	2.6	79.7
4	31.4	16.2	14.6	53.6
5	11.6	19.5	6.1	47.5
6	24.6	14.7	10.5	57.4
7	29.5	20.2	5.3	82.1
Avg.	19.9	16.1	8.8	55.8

1. Endres, J. Future Trends in Low Fat Spreads. *Inform*, 5:1354-1356, (1994)
2. Chrysam, M. Table Spreads and Shortenings. In: Bailey's Industrial Oil and Fat Products, vol. 3, John Wiley and Sons, NY. P. 41-126. (1985)
3. Haumann, B.F. Tools:Hydrogenation, Interesterification. *Inform*, 5:668-678, (1994)
4. Haumann, B.F. Widening Array of Spreads Awaits Shoppers. *Inform*, 9:6-13, (1998)
5. Mag, T.K. Margarine Oils, Blends in Canada. *Inform*, 5:1350-1353, (1994)
6. Moustafa, A. Margarines and Spreads in the United States. In: D.R. Erickson (ed.), *Proceedings World Conference on Edible Fats and Oils*, AOCS Press, Champaign, IL. P. 214-220, (1989)
7. Anonymous. FDA *Trans* Rule Expected Within 60 Days. *Inform*, 10:678-679, (1999)
8. Erickson, D.R. and M.D. Erickson. Hydrogenation and Base Stock Formulation Procedures. In: *Practical Handbook of Soybean Processing and Utilization*, AOCS and United Soybean Board, Champaign, IL. P. 218-238. (1995)
9. Latondress, E.G. Formulation of Products From Soybean Oil. *JAOCs*, 58:185-187, (1981)
10. Dijkstra, A.J. Hydrogenation Revisited. *Inform*, 8:1150-1158, (1997)
11. Neff, W.E., R.O. Adlof, G.R. List and M.A. El-Egaimy. Analysis of Vegetable Oil Triglycerides by Silver Ion High Performance Liquid Chromatography with Flame Ionization Detection. *J. Liq. Chrom.*, 17:3951-3968. (1994)
12. Neff, W.E., G.R. List, and W.C. Byrdwell. Quantitative Composition of High Palmitic and Stearic Acid Soybean Oil Triacylglycerols by Reversed Phase High Performance Liquid Chromatography. *J. Liq. Chrom.*, 22:1649-1662, (1999)
13. Official Methods and Recommended Practices of the American Oil Chemists Society. AOCS, Champaign, IL. (1989)
14. Byrdwell, W.C. and W.E. Neff. Qualitative and Quantitative Analysis of Triacylglycerols Using Atmospheric Pressure Chemical Ionization Mass Spectrometry. In *New Techniques and Application in Lipid Analysis*, AOCS Press, Champaign, IL, pp.45-79, (1997)
15. Neff, W.E. and W.C. Byrdwell. Soybean Oil Triacylglycerol Analysis by Reversed Phase High Performance Liquid Chromatography Coupled With Atmospheric Chemical Ionization Mass Spectrometry. *JAOCs*, 72, 1185-1191, (1995)
16. Neff, W.E., T.L. Mounts, W.M. Rinsch, H. Konishi and M.A. El-Egaimy. Oxidative Stability of Purified Canola Oil Triacylglycerols With Altered Fatty Acid Composition as Affected by Triacylglycerol Composition and Structure. *JAOCs*, 71, 1101-1109, (1994)
17. Deman, L., C.K. Chen and J.M. Deman. Physical and Textural Characteristics of Soft Tub Margarines. *JAOCs*, 68:70-73. (1991)

18. List, G.R., W.E. Neff, R.L. Holliday, J.W. King and R. Holser. Hydrogenation of Soybean Oil Triglycerides: Effect of Pressure on Selectivity. In Press, JAOCS. (2000)
19. Postmus, E., L. Deman and J.M. Deman. Composition and Physical Properties of North American Stick Margarines. Can. Inst. Food Sci. Technol., 22:481-486. (1989)

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Technologies Supporting the Adoption of Biodiesel as an Alternative Fuel

**Thomas A. Foglia*, Kerby C. Jones,
Michael J. Haas, and Karen M. Scott
U.S. Department of Agriculture
Agricultural Research Service
Eastern Regional Research Center
Wyndmoor, PA**



Technologies Supporting the Adoption of Biodiesel as an Alternative Fuel

Thomas A. Foglia*, Kerby C. Jones, Michael J. Haas, and Karen M. Scott

U. S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038

The idea of using vegetable oil as a substitute for diesel fuel was demonstrated by the inventor of the diesel engine, Rudolph Diesel, around the year 1900. Since then, research in this area has continued with various vegetable and animal fat-derived biofuels having been widely tested as alternative diesel fuels (1). To overcome problems (high viscosity and fuel injector fouling) associated with the use of intact triglycerides as diesel fuels, the oil and/or fat is converted to simple alkyl esters (primarily methyl and ethyl esters). Today, "biodiesel" is the term applied to simple alkyl fatty acid esters used as alternatives to petroleum-based diesel fuels.

The relatively high cost of refined oils and fats makes biodiesel produced from these materials more expensive than petroleum-derived diesel fuel. To reduce the cost of biodiesel there has been considerable investigation of the use of lower quality/lower value lipids, such as spent fryer grease and byproducts of edible oil refining, as feedstocks for biodiesel production.

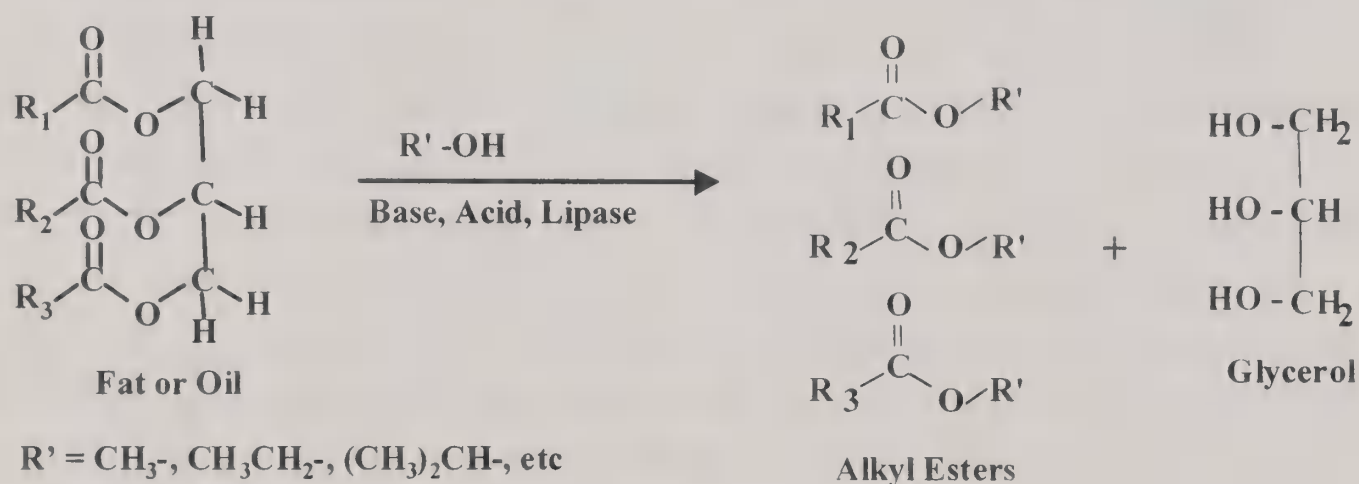
Biodiesel is in commercial production in several countries worldwide. In Europe rapeseed and sunflower oils are the major feedstocks, along with waste grease. In Malaysia, palm oil esters are of interest. In the United States, soybean oil esters feature prominently as do the esters of tallow and spent restaurant oils. For most technical applications, methyl esters are produced because methanol is readily available and relatively inexpensive. In some cases, however, it may be preferable to prepare ethyl esters because ethanol is less toxic than methanol. Moreover, because ethanol can be produced from grain or biomass, ethyl ester biodiesel is a fuel that can be totally derived from renewable resources.

Presently, the driving forces behind the use of biodiesel fuels in the United States are mainly environmental concerns. Vegetable oil- and animal fat-based biodiesel fuels, being alkyl esters, have the following advantages over petroleum diesel fuel: as a neat fuel or in blends with petroleum diesel they; produce less smoke and particulates, have higher cetane numbers, produce lower carbon monoxide and hydrocarbon emissions, and are biodegradable and non-toxic. Conversely, biodiesel fuels present technical challenges of their own, such as low volatility; high pour, cloud, and cold filter plugging points; elevated NO_x emissions under some conditions; and

incomplete combustion. The advantages and disadvantages of fat and oil-derived alkyl ester diesel fuels with respect to fuel properties, engine performance, and emissions have recently been reviewed (2).

Currently, biodiesel is most commonly made by the alkali-catalyzed transesterification of an oil or fat with an alcohol, usually methanol, a process that transfers the glyceride fatty acids from glycerol to methanol, producing fatty acid methyl esters (FAME) and glycerol (Scheme).

SCHEME



After separation of the glycerol and FAME products, the latter are water-washed to remove traces of glycerol, alcohol, and catalyst, before being blended with petroleum diesel or used neat as fuel. The most commonly used blend is 20 volume % in petroleum diesel, which is referred to as a B-20 blend. This is the preferred blend level since the biodiesel component provides a desirable final 2.2 weight % of oxygenate in the B-20 fuel. This level of oxygenate imparts the desired reduction in emissions when using biodiesel, while minimizing the effects of the power reduction that results from the lower energy content of biodiesel compared to petroleum diesel.

Chemical Synthesis of Biodiesel:

Several chemical procedures are available that use various catalysts for the alcoholysis of fats and oils (3). The most commonly used catalysts are alkali hydroxides and alcoholates. When the feedstock has a high free fatty acid (FFA) content, as is common with rendered fats and spent restaurant oils, some operators merely add excess alkali, and subsequently remove the FFA as their insoluble soaps. However, this decreases the final yield of ester, and consumes alkali. As

an alternative, one can conduct an acid-catalyzed reaction, which simultaneously achieves transesterification of the glyceride and esterification of the FFA. However, compared to alkali-catalyzed transesterification, this requires higher reaction temperatures and longer reaction times. More commonly, for FFA-containing feedstocks, a two-step process is used. The first step is the acid-catalyzed esterification of the FFA, followed by neutralization of the acid and alkali-catalyzed transesterification of glycerides. A disadvantage of these chemical procedures is that the catalysts are removed with the side stream containing the coproduct glycerol and cannot be reused. Moreover, spent catalyst removal increases the cost of glycerol purification.

Full conversion of FFA and glycerides in the feedstock to alkyl esters is a necessary feature for any method used to produce biodiesel, since even low residual amounts of these materials reduce the handling and performance characteristics of the fuel. Failure to attain these values during esterification necessitates costly cleanup steps that further increase the cost of the fuel.

Lipase-Catalyzed Biodiesel Synthesis:

Biochemical catalysis, mediated by lipases, offers an alternative route for the esterification of free fatty acids and the transesterification of glycerides. The advantages of lipase catalysis over chemical methods for the production of simple alkyl esters include: room temperature reaction conditions; the potential of catalyst reuse; the ability to esterify both glyceride-linked and unesterified fatty acids in one-step; and production of a glycerol sidestream with minimal water content and little or no inorganic material (4). Bottlenecks to the use of lipases include their relatively high costs compared to inorganic catalysts without effective schemes for their multiple usage and stabilization, inactivation of the lipase by contaminants in the triglyceride feedstocks, and/or substrate inhibition or inactivation, especially with polar short-chain alcohol substrates.

One drawback to the use of esters prepared from refined vegetable oils is the relatively high feedstock cost compared to other triglyceride substrates. A lower cost substrate is tallow. However, since tallow contains lower levels of unsaturated fatty esters and higher proportions of saturated fatty acids, biodiesel prepared from tallow has poor low temperature properties compared to vegetable oil-derived biodiesel (5). One way to partially overcome this difficulty is to prepare branched-chain esters of tallow or tallow vegetable oil ester blends. A study was conducted using different lipases, vegetable oils and tallow, under different conditions to determine the best conditions for preparing biodiesel (6). Several commercially available lipases

were screened for their abilities to transesterify the triacylglycerols (TAG) of olive, soybean, rapeseed, and tallow with short chain alcohols (methyl and ethyl). Ninety-percent conversion to esters was achieved. The use of fuel grade ethanol instead of absolute ethanol gave comparable conversions. Transesterification of tallow with secondary alcohols (isopropyl) resulted in yields in excess of 95 %, with minimum production of partial glycerides. By modification of these conditions, similar conversions also could be obtained for both the methanolysis and isopropanolysis of soybean and rapeseed oils (7).

Enzymatic conversion of greases to biodiesel:

Research on the low temperature properties and diesel engine performance of selected mono-alkyl esters derived from tallow and spent restaurant grease strongly suggested that ethyl esters of grease (i.e., ethyl greasate) might be an excellent source of biodiesel (8). Ethyl esters of grease have low-temperature properties closely resembling those of methyl soyate, the predominant form of biodiesel currently marketed in the United States. Results obtained from diesel engine performance and emission tests for 20% blends of ethyl greasate in No. 2 petroleum diesel fuel were comparable to the 20 % blends of methyl soyate. The ethyl greasate used in the tests was synthesized enzymatically because low-value lipids, such as waste deep fat fryer grease, usually have high levels of free fatty acids (8% or greater) and both these and the glyceride-linked fatty acids are effectively converted to simple alkyl esters by lipases.

Conversion of soapstocks to biodiesel:

One step in the refining process for edible vegetable oils involves the addition of water and alkali to the crude oil, causing precipitation of a semisolid material known as "soapstock". Although soapstock is used to prepare several products, it is nevertheless considered to be an underused byproduct, and there is considerable interest in finding ways to convert soapstocks, which contain free fatty acids, glycerides, and phospholipids, to industrial useful products. To address this goal, a partial enzymatic process was devised that directly converted soapstocks to fatty esters suitable for biodiesel production (9). In this two-step, solvent-free process, dried soapstock was first treated with alcohol and alkali to promote transesterification of the glyceride and phosphoglyceride fatty esters. In the second step, enzymatic catalysis was employed to esterify the free fatty acids in the mixture. However, only about two-thirds of all fatty acids

present in the starting material was converted to methyl esters by this procedure. More recently, a two-step chemical procedure, which uses inexpensive inorganic reagents, was developed that gives high yields of FAME suitable as a biodiesel fuel (10). In the first step the ester-linked fatty esters in soapstock are released by saponification, yielding fatty acid soaps. In the second step the soaps are converted to FFA and esterified to FAME using an acid catalyst in methanol.

Analytical methods of analysis for biodiesel:

It is important to determine the degree of conversion of fatty acids to esters in any biodiesel synthesis. It also is advisable to establish the degree of purity and the type of contaminants present in a biodiesel prior to its use. Accordingly, methods have been developed to monitor the composition of the products of the transesterification reaction and assess the quality of the biodiesel produced by either chemical or enzymatic procedures.

One laboratory measured glyceride transesterification mixtures prepared as biodiesel by thin-layer chromatography with flame-ionization detection (TLC/FID) (11). This analytical technique, although quantitative, was time consuming, difficult to conduct, and labor intensive. The transesterification reaction mixtures subsequently were analyzed by capillary gas chromatography (GC), which detected and quantitated esters, triglycerides, diglycerides, and monoglycerides in one run (12). Since it is important to determine the total amount of residual glycerol (bound plus free) in biodiesel, the capillary GC approach was subsequently augmented to include the determination of free glycerol (13).

Analysis of biodiesel by GC requires that the hydroxyl groups of the glycerides and glycerol be derivatized. A high performance liquid chromatographic (HPLC) method has been developed for analyzing transesterified fats and oils (14). Advantages of the method are that derivatization of the sample is not required, analysis time is under 30 min, and all neutral lipid classes, including alkyl esters, free fatty acids, triglycerides, 1,2- and 1,3-diglycerides and 1(2)-monoglycerides, are readily quantitated. The method, however, does not measure free glycerol.

A rapid analytical method that is particularly suitable for continuous analysis is near-infrared spectroscopy. The infrared spectra of triacylglycerols and their corresponding methyl esters are similar. However, with the recent availability of fiber-optic probes for near infrared spectroscopy, it is now possible to monitor the degree of alcoholysis of fats and oils directly in a reaction medium (15).

Conclusion: From the foregoing, it is demonstrated that recently developed lipase- and chemical-catalyzed transesterification procedures are potentially viable methods for the production of alkyl esters from vegetable oils, tallow, greases and soapstocks. Work is ongoing to maximize conversions and scale-up reactions to provide sufficient quantities of these esters for determination of their cold-temperature and fuel properties.

References

1. G Knothe, RO Dunn, MO Bagby. Biodiesel: The use of vegetable oils and their derivatives as alternative diesel fuels, in *Fuels and Chemicals from Biomass*, B. C. Saha and J. Woodward, editors, Am. Chem. Soc. Symposium Series, no 666, ACS, Washington, DC, 1997, pp. 178-208.
2. MS Graboski, RL McCormick. Combustion of fat and vegetable oil derived fuels in diesel engines. *Prog Energy Combust Sci* 24:125-164, 1998.
3. G Vicente, A Coteron, M Martinez, J Aracil. Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. *Ind Crops and Prod* 8:29-35, 1998.
4. Y Shimada, Y Watanabe T Samukawa, A Sugihara, H Noda, H Fukuda, Y Tominaga. Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. *J. Am Oil Chem Soc* 76:789-793, 1999.
5. TA Foglia, LA Nelson, WN Marmer, GH Knothe, RO Dunn, MO Bagby. Improving the properties of vegetable oils and fats for use as biodiesel: Proceedings of the World Conference on Oilseed and Edible Oil Processing. AOCS Press, Champaign, IL, 1996.
6. LA Nelson, TA Foglia, WN Marmer. Lipase-catalyzed production of biodiesel. *J Am Oil Chem Soc* 73:1191-1195, 1996.
7. TA Foglia, LA Nelson, WN Marmer. Production of biodiesel, lubricants, and fuel and lubricant additives. United States Patent 5,713,965, 1998.
8. W-H Wu, TA Foglia, WN Marmer, RO Dunn, CE Goering, TE Briggs. Low-temperature properties and engine performance evaluation of ethyl and isopropyl esters of tallow and grease. *J Am Oil Chem Soc.* 75:1173-1178, 1998.

9. MJ Haas, KM Scott. Combined nonenzymatic-enzymatic method for the synthesis of simple alkyl fatty acid esters from soapstock. *J Am Oil Chem Soc*. 73:1393-1401. 1996.
10. MJ Hass, S Bloomer, KM Scott. The simple, high-efficiency synthesis of fatty acid methyl esters from soapstock. *J. Am Oil Chem Soc* in press, 2000.
11. B Freedman, EH Pryde, WF Kwolek. Thin layer chromatography/flame ionization analysis of transesterified vegetable oils. *J Am Oil Chem Soc* 61:1215-1220, 1984.
12. B Freedman, WF Kwolek, EH Pryde. Quantitation in the analysis of transesterified soybean oil by capillary gas chromatography. *J Am Oil Chem Soc* 63:1370-1375, 1986.
13. C Plank, E Lorbeer. Simultaneous determination of glycerol and mono-, di-, and triglycerides in vegetable oil methyl esters by capillary gas chromatography. *J Chromatogr A* 697:461-468, 1995.
14. TA Foglia, KC Jones. Quantitation of neutral lipid mixtures using high performance liquid chromatography with light scattering detection. *J Liq Chrom & Rel Technol* 20:1829-1838. 1997.
15. G Knothe. Rapid monitoring of transesterification and assessing biodiesel fuel quality by near-infrared spectroscopy using a fiber-optic probe. *J Am Oil Chem Soc* 76:795-800. 1999.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Identification of Genes for Enzymes Involved in the Synthesis of Tung Oil

**H. Shepherd¹, J. Dyer¹, F. Tang², D. Chapital¹,
D. Shih², and A. Pepperman¹**

**¹ Southern Regional Research Center, ARS, USDA
New Orleans, LA**

**²Department of Biological Sciences, LSU
Baton Rouge, LA**



1994

1994

1994

1994

1994

1994

1994

1994

1994

1994

Identification of Genes for Enzymes Involved in the Synthesis of Tung Oil

H. Shepherd, J. Dyer, F. Tang, D. Chapital, D. Shih, A. Pepperman

Southern Regional Research Center, ARS, USDA, New Orleans, LA (H.S., J.D., D.C., A.P.)
and Department of Biological Sciences, LSU, Baton Rouge, LA (F.T., D.S.)

Tung oil is pressed from the seeds of the tung tree (*Aleurites fordii*) and is used in formulations of inks, paints, and finishes because of its rapid drying qualities. Currently, almost all tung oil used in the U.S. is imported, with the price and quality highly variable. Our research is aimed at finding genes responsible for giving tung oil its unique properties. These genes, when identified and isolated, could then be transferred to a microbial system, such as yeast. Growth of yeast on vegetable oils, such as cottonseed or soybean oil, could convert the domestic oils into value-added drying oils.

Since a key component of tung oil is an unsaturated fatty acid known as eleostearic acid, we have focused on identification of desaturation genes, which are involved in synthesis of unsaturated fatty acids, including linoleic, linolenic, and eleostearic. We have identified genes for three fatty acid desaturase enzymes which are similar to those which produce linolenic acid in other plants. The enzymes encoded by these genes have features which are common to fatty acid desaturase enzymes from other plants, but with distinctive differences from each other and from the enzymes of other plants. We are currently studying the function of these genes in tung oil biosynthesis and performing experiments to identify additional desaturases involved in the synthesis of tung oil. The time of expression of the genes in tung trees is also being analyzed.

To isolate the genes, we constructed a tung seed cDNA library using RNA from the time of oil production. The library was screened using primers based on sequences of known fatty acid desaturase genes to identify genes which may be involved in tung oil biosynthesis. Three fatty acid desaturase genes of the omega-3 type have thus far been found to be expressed in tung seeds: one targeted to the endoplasmic reticulum (TnDES1, GenBank accession number AF047172); one targeted to chloroplast (TnDES2, accession number AF200717); and one with homology to plastid proteins (TnDES3, accession number AF061027). Omega-3 fatty acid desaturases introduce the third double bond into 18:3 fatty acids, such as linolenic acid, which are important components of plant membranes and plant oils (Yadav et al., 1993).

Clone TnDES1 encodes a polypeptide of 387 amino acids with an endoplasmic reticulum retention sequence (lysine at the 3rd and 5th residues from the C terminus) (Nakai and Kanehisa, 1992). BLAST analysis (Altschul et al., 1997) reveals close homology to previously reported proteins from *Pelargonium* (AF020204, omega-3 desaturase, 67% amino acid identity, 76% similarity), and *Brassica napus* (P46311, omega-3 fatty acid desaturase, 66% identity, 75%

similarity).

Clone TnDES2 (Shepherd et al., 2000) contains an open reading frame encoding a polypeptide of 451 amino acids. Comparison to tung desaturase 1 (TnDES1) (AF047172) showed 56% identity and 73 % similarity at the amino acid level. The highest amino acid sequence homology was to chloroplast-type omega-3 desaturases identified in sesame cotyledon (P48620) with 73 % identity, 91% similarity), and in castor bean seed (P48619), 70% identity, 89% similarity. A putative chloroplast transit peptide was also identified (residues 1-68) beginning with the met-ala dipeptide which is typically found at the start of chloroplast transit peptides. These similarities suggest that TnDES2 encodes a chloroplast-type omega-3 fatty acid desaturase.

Clone TnDES3 (Tang et al., 1999) is a partial gene sequence which encodes a polypeptide of 437 amino acids, but lacking a portion of the N terminus. The amino acid sequence has 72% identity to TnDES1 and 82% similarity; and 65% identity to TnDES2 with 80% similarity. The deduced amino acid sequence of TnDES3 showed greatest homology to plastid omega-3 desaturases of *Sesamum indicum* (U25817, 77% identity, 83% similarity), castor bean (P48619, 75% identity, 83% similarity). These characteristics suggest that the TnDES3 cDNA encodes a plastid-type omega-3 fatty acid desaturase.

The deduced amino acid sequences also reveal three highly conserved histidine boxes (at amino acid positions 170-174, 206-210, and 373-377), whose sequence and spacing are well conserved among membrane-bound fatty acid desaturases (Sakamoto et al., 1994). These regions are thought to be involved as the active site or in metal ion binding (Yadav et al., 1993).

There is a high degree of homology in all omega-3 type fatty acid desaturases in the middle of the protein where the active and other structural sites are located, since all do essentially the same reaction (Yadav et al., 1993; van de Loo and Somerville, 1994). This is also true of the three reported here, as revealed by CLUSTAL analysis (Thompson et al., 1994), with identical residues at 49% of the sites (marked by * in figure) and strongly similar amino acids at an additional 14% (marked by : in figure). Most differences are at the N and C termini of the protein where targeting and other regulatory and control sites are located. (See Figure)

REFERENCES

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acid Res* **25**: 3389-3402
- Nakai N, Kanehisa M (1992) A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics* **14**: 897-911
- Sakamoto T, Wada H, Nishida I, Ohmori M, Murata N (1994) Identification of conserved domains in the delta 12 desaturases of cyanobacteria. *Plant Mol Biol* **24**: 643-650

Shepherd HS, Dyer JM, Tang F, Shih DS, Pepperman AB (2000) Nucleotide sequence of a cDNA clone of a plastid-type omega-3 fatty acid desaturase from tung (*Aleurites fordii*) seeds (PGR 00-009) Plant Physiol **122**: 291

Tang F, Dyer JM, Lax AR, Shih DS, Chapital DC, Pepperman AB (1999) Nucleotide sequence of a cDNA clone for omega-3 fatty acid desaturase from *Aleurites fordii* seeds. Plant Physiol **119**: 364

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, **22**: 4673-4680

van de Loo FJ, Somerville C (1994) Plastid omega-3 desaturase cDNA from *Ricinus communis*. Plant Physiol **105**: 443-444

Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Perez-Grau L, Kinney AJ, Hitz WD, Booth JR, Schweiger B, Stecca KL, Allen SM, Blackwell M, Reiter RS, Carlson TJ, Russell SH, Feldmann KA, Pierce J, Browse J (1993) Cloning of higher plant omega-3 fatty acid desaturases. Plant Physiol **103**: 467-476

FIGURE

Amino acid sequence comparison of three tung omega-3 fatty acid desaturase genes

	10	20	30	40	50
TNDES3_TUNG					
TNDES1_TUNG	-----	-----	-----	-----	-----
TNDES2_TUNG	MAVWALSDCGIRPLPKIYSKPRLAFTSNNPQPTKPPIPRPELRNGSSFKL				
Prim.cons.	MA2222222222222222SK2RL23T3333KDTK2PI3NG22R2GSSFKL				
	60	70	80	90	100
TNDES3_TUNG					
TNDES1_TUNG	-----	-----	-----	-----	-----
TNDES2_TUNG	SSAGLRDKWVNL SAPLRGGLVEEDEDNFEGNRVISIDESGGEDFDPDAP				
	..:	::*:. *			::*:. *
	110	120	130	140	150
TNDES3_TUNG					
TNDES1_TUNG	PPFKLSDIRAAIPKHCWVKDPWRMSYVVRDVAVVFGLA AAAAYLNNWIV				
TNDES2_TUNG	PPFNIGQIRAAIPKHCWKNPWRSLTYVFRDVVVVFALAAAFYFNSWLF				
	PPDTLADIRAAIPKHRWTKNPEISLSYVVRNVAVVSGLA AAVAAYFNDWAF				
	** .:.*:*	***** *:.*:*	*:.*:*. *:.*:*	*.***.* *:.*:*	.:***.*
	160	170	180	190	200
TNDES3_TUNG					
TNDES1_TUNG	WPLYWAAQGTMFWALFVLGHDCGHGSFSHNPKLNSVVGHLHSSILVPIYH				
TNDES2_TUNG	WPLYWFAQGTMFWAI FVLGHDCGHGSFSNSSLNNVVGHLHSSILVPIYH				
	WSPYWICQGTMLSALFVLGHDCGHGSFSNNPNLDSVVGHLHHSILVPIYH				
	*. ** .:***.*: *	:***** *:.*:*	*:.*:*. *:.*:*	*.***.* *	*****
	210	220	230	240	250
TNDES3_TUNG					
TNDES1_TUNG	GWRISHRTHHQNHGHVENDES WQPLSEKIFRSLDYMTRTLRFTVPSPMLA				
TNDES2_TUNG	GWRISHRTHHQNHGVEKDES WVPLPEKIYKEMDLSTRILRYSVPLPMFA				
	GWRISYRTHYRHHQHAENDES WHPLSEKIYKGLNNVTRTLRLSLPFSLLA				
	*****:***.:*: *	:.*:***** **.*:***: :	** ** *:.*:*	:.*:*	.:.*:*
	260	270	280	290	300
TNDES3_TUNG					
TNDES1_TUNG	YPFYLNWRSPGKTGSHFHPDSDLFGPNERKD VITSTVCWTAMAALLVGLS				
	LPFYLWWRSPGKEGSHFNPNSDFFAPHERKAVLTSNFCFSIMALLLLYSC				

1920年1月1日
（昭和五年）

（昭和五年）

昭和五年
（昭和五年）

（昭和五年）

昭和五年
（昭和五年）

昭和五年
（昭和五年）

（昭和五年）

昭和五年
（昭和五年）

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Gossypol Isomers in Seed of Upland (*Gossypium hirsutum*) and Pima (*Gossypium barbadense*) Cottons

M.C. Calhoun, B.C. Baldwin, and S.W. Kuhlmann
Texas Agricultural Experiment Station
The Texas A & M University System
San Angelo, TX 76901



49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Early Harvest Can Reduce Aflatoxin Contamination of Cottonseed

C. H. Bock and P. J. Cotty
Research Plant Pathologists
Food and Feed Safety Research
Southern Regional Research Center, ARS
United States Department of Agriculture
New Orleans, LA



Early Harvest Can Reduce Aflatoxin Contamination of Cottonseed

C. H. Bock and P. J. Cotty
Research Plant Pathologists
Food and Feed Safety Research
Southern Regional Research Center
Agriculture Research Service
United States Department of Agriculture
P.O. Box 19687
New Orleans, LA 70179

Summary

A commercial gin cooperative in western Arizona segregated cottonseed at ginning by field of origin in 1995 and 1996. Records of harvest date, gin date, and cultivar were maintained and the crop from each field was sampled and analyzed for aflatoxin content according to Arizona Commercial Feed law. Date of harvest was the most important observed influence on aflatoxin content. Earlier harvested cottonseed was the most likely seed to have acceptable aflatoxin content. Regression analysis indicated significant relationships between harvest date and aflatoxin content in both years. Overall, 89% and 79% of seed lots exceeded 20 ppb in 1995 and 1996, respectively. Of all crops harvested before September 7th (Julian Day 250), 86% had <20 ppb aflatoxin and only 5% had >300 ppb. In contrast, no crop harvested after October 27th (Julian Day 300) had <20 ppb aflatoxin, and 72% had >300 ppb aflatoxin. Both transgenic *Bt* and non-*Bt*

cottonseed were contaminated. The mean aflatoxin content of *Bt* cottonseed in 1996 was 413 ppb and that of non-*Bt* was 303 ppb. Similar harvest date trends were observed in subsequent years and aflatoxin contamination of *Bt* cottonseed was also observed in the 1997, 1998, and 1999 crops. Bolls remain susceptible to contamination after they mature and open. Aflatoxin contamination of open bolls is favored by exposure to warm, moist conditions. To reduce the risk of aflatoxin contamination, cotton crops should be harvested as early as economically feasible. Irrigation should be reduced late in the season and, when possible, irrigation should be discontinued after the first bolls open.

INTRODUCTION

Aflatoxin contamination of cottonseed is caused by the fungus *Aspergillus flavus*. Aflatoxins are chemical compounds that have toxic and carcinogenic effects on humans and other animals (17). Many countries regulate the quantity of aflatoxins allowed in foods and feeds (17).

When cottonseed is crushed for oil, the remaining meal competes with other vegetable meals in the livestock feed market. During crushing, aflatoxin is concentrated about two-fold in the meal. In the USA, both cottonseed and cottonseed meal may be sold at a premium for dairy feed only if the aflatoxin content does not exceed 20 ppb (17). Seed and meal exceeding 300 ppb aflatoxin may not even be used as feed for mature cattle.

In the southwestern United States, aflatoxin contamination of cottonseed frequently occurs. Irrigation can influence aflatoxin contamination, particularly if

continued into the harvest season (5). Indeed, field plot and laboratory observations suggest later harvested crops tend to have greater contamination (8). The cotton plant is indeterminate; the first bolls to mature and open are exposed to the environment while later bolls are developing. Bolls produced closer to the ground frequently contain the greatest aflatoxin contamination (1). These bolls are both the first to open and the closest to irrigation water, dew, and rain run-off (15).

Contamination occurs in two phases (5): the first occurs prior to boll maturity and the second after maturity. Insect damage, particularly pink boll worm damage (*Pectinophora gossypiella* Lepidoptera: Gelechiidae) is associated with the first phase. Bolls infected during this phase frequently produce seed that exhibits blue-green-yellow-fluorescence (BGYF) on the lint and linters (1, 9). The second phase occurs after seed maturity, and includes direct infection of the seed by *A. flavus*. This process is favored by warm, moist conditions (2, 5). Contamination of some bollworm-resistant *Bt* cottonseed lots in 1995 was attributed to the second phase of contamination (8). During the second phase, the aflatoxin content of seed initially infected with *A. flavus* as a result of insect injury (during the first phase) may also increase. Thus, harvest date appears to be an important factor influencing final aflatoxin content.

During 1995 and 1996, we collaborated with a commercial gin in order to analyze precisely the impact of harvest date on aflatoxin content. The result is a clear association between harvest date and cottonseed aflatoxin content that

should be considered when developing integrated management systems for the management of aflatoxin contamination.

MATERIALS AND METHODS

Cottonseed from a gin cooperative in the Mohawk Valley in western Arizona was assayed for aflatoxin on a field-by-field basis. A total of 38 and 101 fields were assayed in 1995 and 1996, respectively. The aflatoxin contents reported here were determined as mandated by the Arizona Commercial Feed Law and were the values used for commerce. Aflatoxin contents of seed lots sent to oil mills were determined by the mill using official methods (seed sent to oil mills typically is not analyzed by the gin). The aflatoxin values were averaged where multiple truckloads of seed were harvested from a single field. Cottonseed lots that were not sent to an oil mill were sampled at the gin for subsequent aflatoxin analysis at a commercial laboratory. Samples were taken from the processing line immediately after ginning using a robotic in-line sampler. For each field, small samples (75-125 g) were taken at regular intervals and combined to make a composite sample (12-18 kg per field). Law mandates that no more than 100 tons of cottonseed may be sampled per analysis; therefore, maximum field size was less than 100 acres. In 1995, growers were asked to mark modules and indicate field of origin. In 1996, growers were also asked to supply harvest date and cultivar. In both years, all seed lots ginned were included except those intended for use as planting seed for which aflatoxin content was not analyzed.

RESULTS

In both years, aflatoxin content ranged from <20 to >2000 ppb. The percent of seed lots with aflatoxin contamination below the maximum level allowed for dairy use (20 ppb) was 11% in 1995 and 21% in 1996 (Table 1). Contamination exceeded 300 ppb in 46% and 30% of cottonseed lots ginned in 1995 and 1996, respectively.

Aflatoxin contents of cottonseed lots were significantly correlated with gin date in both 1995 and 1996, with aflatoxin increasing with later ginning (Figure 1). The relationship with the log transformed data for individual cottonseed lots was linear in both 1995 ($R^2=0.49$) and 1996 ($R^2=0.55$) and highly consistent between years.

Mean weekly aflatoxin content increased until the week ending Julian Day 308 in 1995 and 1996 (Figure 2). After this date, mean weekly aflatoxin levels were based on fewer than three values and the trend was less clear.

Transgenic *Bt* and non-*Bt* cottons were grown in both years. In 1995 only three *Bt* cottonseed lots were available for analysis (Table 2). Aflatoxin ranged from 600 to 7,000 ppb, and among the non-*Bt* from 2 to 2,375 ppb (37 lots). In 1996, eight transgenic *Bt*-cotton crops were analyzed during the period Julian Day 280 to Julian Day 337 and the mean aflatoxin content was 413 ppb (range 92-1,000 ppb), while that of the 34 non-*Bt* cottonseed lots ginned during the same period was 598 ppb (range 27-2,200 ppb).

DISCUSSION

Harvest date has a major impact on aflatoxin contamination of cottonseed. Cottonseed harvested later in the season has a significantly greater risk of severe and unacceptable aflatoxin contamination. This confirms field plot observations on cotton (5) and corn (11). However, the effect of harvest date in the current study exceeds what might be anticipated from the field plot studies.

Programs directed at limiting aflatoxin contamination of cottonseed need to consider potential harvest date influences. In addition to minimizing the risk of aflatoxin contamination, early harvest reduces overwintering insect pests (10) and lint weathering (thus improving lint grade, 16). Previous observations suggested that once harvested, cotton should be ginned in a timely manner (3, 14). In the current study, ginning closely followed harvest date. Late season irrigation can provide adequate moisture for fungal activity and increased aflatoxin production (15). However, weather observations made during the current study (data not shown) suggest that even in the absence of irrigation, sufficient moisture (in the form of dew or high relative humidity) is available to drive aflatoxin increases.

In the current study, a commercial gin segregated cottonseed lots on a field-by-field basis. At most gins, seed from different fields are co-mingled. Segregation can result in increased numbers of seed lots with acceptable aflatoxin content, and may be an additional cost effective aflatoxin management tool. In this study, segregation resulted in acceptable seed lots when mean aflatoxin contents exceeded 20 ppb between Julian Day 244 and Julian Day 273 in 1995, and between Julian Day 221 and Julian Day 267 in 1996.

Early harvest will reduce the second phase of contamination by reducing the incidence and severity of infections in open bolls. Once seed are colonized by an aflatoxin producing strain of *A. flavus*, the level of contamination will increase with time, provided there is sufficient moisture and warmth for the fungus to grow (5). In Arizona, bolls may mature and open over several months (boll opening depends on date of flowering, canopy position and heat unit accumulation); therefore, both the first and second phases of contamination may occur simultaneously on a single plant.

It was initially thought that transgenic *Bt* cottons resistant to the pink bollworm would be largely resistant to aflatoxin contamination. Early field plot studies under high pink bollworm pressure supported this (4, 8). However, in 1995, the first observation of problems with aflatoxin contamination of *Bt* cottonseed were made (8). In the current study, transgenic *Bt* cottons had aflatoxin contents similar to non-*Bt* cottons. All varieties examined had seed lots exceeding 20 ppb.

Resistance of *Bt* cotton to insect pests reduces the cost of delaying harvest; thus, farmers may hold *Bt* cotton crops in the field longer (7), and as a result, increase the risk of severe aflatoxin contamination.

Early harvest reduces the risk of aflatoxin contamination. However, growers often receive a gin average price for their seed. Thus, growers harvesting early receive the same price as those harvesting late. Greater incentive to growers to harvest early may be gained by giving a monthly or weekly average price based on harvest date. However, if the proportion of the crop harvested early increases, so will the period between harvest and ginning. When ginning is delayed, the

importance of harvesting a dry crop, proper module construction, and module tarping increases (3, 14). This highlights the need for an integrated approach to the management of aflatoxin contamination.

ACKNOWLEDGEMENTS

This is an abbreviated version an article that appeared in the journal Plant Disease (1999, volume 83, pages 279 to 285). Additional information has been added to improve the utility of the article. We thank Carol Morgan, Fred Richard, Harriet Conner and the entire Growers Mohawk Gin community for time and resources invested in this study. Bryan Vinyard provided advice on the statistical analyses.

References

1. Ashworth, L. J., Jr., and McMeans, J. L. 1966. Association of *Aspergillus flavus* and aflatoxins with a greenish yellow fluorescence of cotton seed. *Phytopathology* 56:104-1105.
2. Ashworth, L. J., Jr., McMeans, J. L., and Brown, C. M. 1969. Infection of cotton by *Aspergillus flavus*: Time of infection and the influence of fiber moisture. *Phytopathology* 59:383-385.
3. Batson, W. E., Caceres, J., Cotty, P. J., and Isakeit, T. 1997. Aflatoxin levels in cottonseed at weekly intervals in Arizona, Mississippi and Texas modules. Pp. 116-118. In Volume 1, Proceedings, Beltwide Cotton Conference, January 6-10 1997, New Orleans, Louisiana.
4. Berberich, S. 1995. Is cotton containing the bollguard safe? *Oil Mill Gazetteer* 101:18-19.
5. Cotty, P. J. 1991. Effect of harvest date on aflatoxin contamination of cottonseed. *Plant Disease* 75:312-314.

6. Cotty, P. J. 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. *Phytopathology* 84:1270-1277.
7. Cotty, P. J. 1997. Update on methods to prevent aflatoxin formation. *The Oilmill Gazetteer* 103:33-38.
8. Cotty, P. J., Bock, C. H., Howell, D. R., and Tellez, A. 1997. Aflatoxin contamination of commercially grown transgenic *Bt* cottonseed. Pp. 108-110. In Volume 1, 1997 Proceedings, Beltwide Cotton Conference, January 6-10 1997, New Orleans, Louisiana. National Cotton Council of America, Memphis, Tennessee.
9. Cotty, P. J., and Lee, L. S. 1989. Aflatoxin contamination of cottonseed: Comparison of pink bollworm damaged and undamaged bolls. *Trop. Sci.* 29:273-277.
10. Henneberry, T. J., Bariola, L. A., and Russell, T. E. 1978. Pink bollworm: chemical control in Arizona and relationship to infestations, seed damage, and aflatoxin in cottonseed. *J. Econ. Entomol.* 71:440-448.
11. Jones, R. K., Duncan, H. E., and Hamilton, P. B. 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology* 71:810-816.
12. Lee, L. S., Lee, L. V. Jr., and Russell, T. E. 1986. Aflatoxin in Arizona cottonseed: field inoculation of bolls by *Aspergillus flavus* spores in wind-driven soil. *J. Am. Oil Chem. Soc.* 63:530-532.
13. Park, D. L., Lee, L. S., Price, R. L., and Pohland, A. E. 1988. Review of the decontamination of aflatoxin by ammoniation: current status and regulation. *J. Assoc. Off. Anal. Chem.* 71:685-703.
14. Russell, T. E. 1985. Effect of modular storage of Arizona seed cotton on levels of aflatoxin in seed. *J. Am. Oil Chem. Soc.* 62:515-517.
15. Russell, T. E., Watson, T. F., and Ryan, G. F. 1976. Field accumulation of aflatoxin in cottonseed as influenced by irrigation termination dates and pink bollworm infestation. *Appl. Environ. Microbiol.* 31:711-713.
16. Turner, J. H., Jr., Worley, S., Jr., Ramey, H. H., Jr., Hoskinson, P. E., and Stewart, J. M. 1979. Relationship of week of flowering and parameters of boll yield in cotton. *Agron. J.* 71:248-251.

Table 1. Number cottonseed lots detected in the current study with various concentrations of aflatoxins

Aflatoxin content (ppb)	Year		
	1995	1996	1995+96
0-19.9	4 (11) ^a	21 (21)	25 (18)
20-300	15 (43)	50 (49)	65 (48)
>300	16 (46)	30 (30)	46 (34)
Total	35	101	136

^a Values in parentheses are the percent seed lots in each category.

Table 2. Mean aflatoxin content of transgenic-*Bt* cotton and non-*Bt* cottons harvested in the Mohawk Valley in 1995 and 1996

		Year				
		1995			1996	
		Aflatoxin (ppb)		Fields (#)	Aflatoxin (ppb)	
Variety type	Fields (#)	Mean	Range		Mean	Range
<i>Bt</i>	2	3,700	600-7,000	8	413	92-1,000
Non- <i>Bt</i>	37	525	2-2,375	34	598	27-2,200

^a For 1995, all non-*Bt* cotton fields studied are included. For 1996 only those non-*Bt* cotton fields ginned during the period during which the *Bt* cotton was ginned are included.

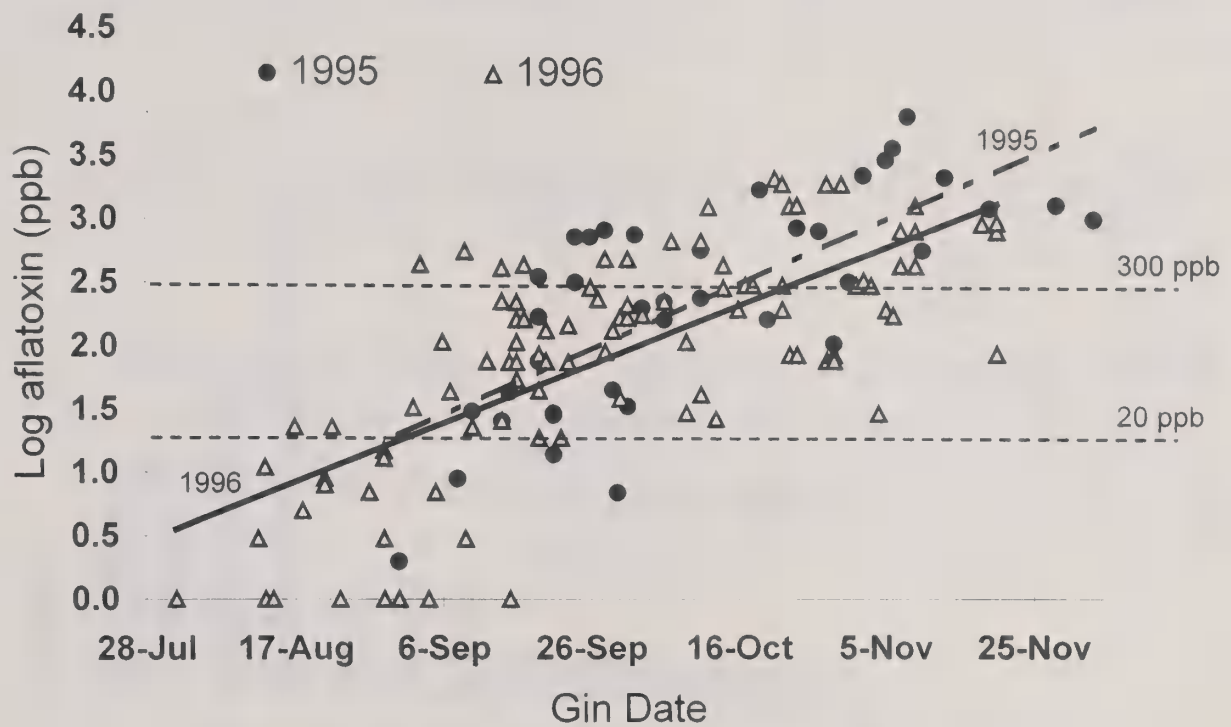


Figure 1. Relationship between gin date and aflatoxin content of cottonseed ginned by one gin in the Mohawk Valley of Arizona. Harvest date and gin date were very closely related at this gin.

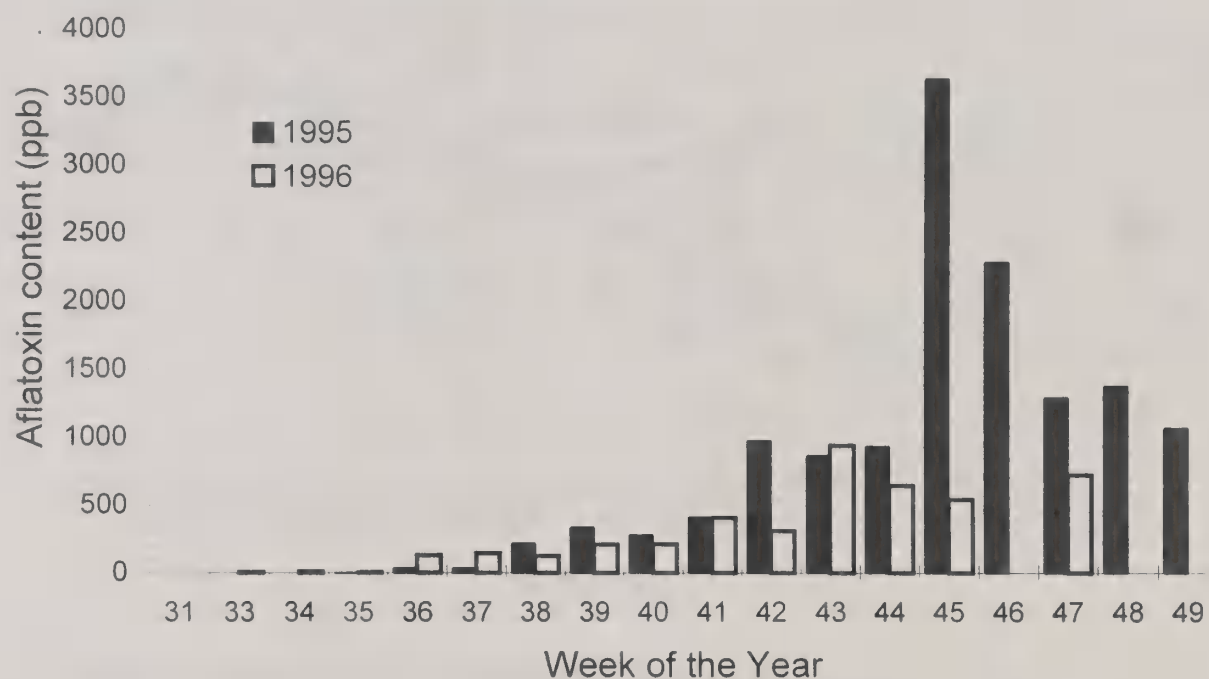


Figure 2. Mean weekly aflatoxin content of cottonseed from one gin in southwestern Arizona during 1995 and 1996.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Domestic Production of Castor Oil

**Thomas A. McKeon, Q. Grace Chen, Karen M.
Lew, Allan E. Stafford, and Jiann-Tsyh Lin**
USDA-ARS Western Regional Research Center
Albany, CA



DOMESTIC PRODUCTION OF CASTOR OIL

Thomas A. McKeon, Q. Grace Chen, Karen M. Lew, Allan E. Stafford and Jiann-Tsyh Lin
USDA-ARS Western Regional Research Center, Albany, CA 94710

Introduction

Vegetable oils are an important part of the diet and are also a source of fatty acids that are used to produce industrial chemicals. We are using transgenic approaches to develop a domestic crop that produces castor oil. These plants will be value-enhanced crops that decrease dependence on petroleum and serve as renewable resources supporting development of the bio-based chemical industry.

The castor bean produces an oil of unique composition: up to 90% of the fatty acid content is ricinoleate (12-hydroxy-oleate). As a result of its physical and chemical properties, castor oil and products derived from it are used for numerous bio-based products, including lubricants, paints, coatings, plastics and anti-fungals (Table 1) (1). However, the presence of the toxic protein ricin and highly allergenic storage proteins are serious obstacles to growing and processing castor in the U.S. As a result, all of the castor oil used in the U.S. is imported, approximately 110 million lbs. per year. We are employing two complementary approaches to provide a domestic source of castor oil.

We have identified the enzymatic steps that are key to the biosynthesis of castor oil. Several of the genes for these enzymes have been cloned from castor or other oilseeds and these are being tested to develop transgenic plants that produce an oil with high ricinoleate content. While the model plant *Arabidopsis* is our initial test system, these genes will ultimately be introduced into soybean.

Complementary to this approach, improvement of castor to diminish the hazardous components would support domestic production of castor. The ability to genetically transform castor would greatly benefit attempts to develop low toxin and low allergen varieties of castor, as well as improve agronomic traits of castor plants. We have developed a successful transformation system for castor and will describe the potential for improving castor to become a domestic crop.

Biosynthesis of Castor Oil

Results of our research on castor oil biosynthesis were described in these Proceedings last year (2), and are summarized here. We have used HPLC analysis of castor bean microsomal incubations to follow the hydroxylase reaction and the movement of ^{14}C -oleate and ricinoleate through phospholipid into triacylglycerol (3). These results have elucidated the basis for high incorporation of ricinoleate and exclusion of oleate from triacylglycerols, enabling us to identify genes that can be used to engineer high ricinoleate production in transgenic plants. The steps leading to high production of ricinoleate and incorporation into triacylglycerol include:

- a) the lyso-phosphatidylcholine acyltransferase (LPCAT) which transfers oleate from oleoyl CoA into the sn-2 position of PC for hydroxylation.
- b) the oleoyl-12-hydroxylase which hydroxylates the sn-2 oleate to form sn-2 ricinoleoyl-PC for hydrolysis.

- c) phospholipase A₂ which preferentially removes ricinoleate from the sn-2 position and releases lyso-PC for reincorporation of oleate by LPCAT; the free fatty acid is preferentially incorporated into ricinoleoyl-containing diacylglycerol.
- d) the diacylglycerol acyltransferase (DAGAT) preferentially incorporates ricinoleate to form diricinoleins and triricinolein.

Table 1
Products Derived from Castor Oil

Lubricants
-Lithium grease
-Heptanoate esters for engines
Coatings
-Nonyellowing drying oil
-Low VOC oil-based paints
Surfactants
-Turkey Red Oil
Plasticizers
-Blown oil, used in polyamides, rubber
-Heptanoates, for low temperature uses
Cosmetics
-Lipstick
Pharmaceuticals
-Laxative
Polymers
-Polyesters, from sebacic acid
-Polyamides, Nylon 11, Nylon 6,10
-Polyurethanes
Attractants & Perfumes
-Odorants include 2-octanol, heptanal and undecenal
Fungicides
-Undecenoic Acid and Derivatives

Castor Transformation

As mentioned, the cultivation is problematic due to castor's high allergen and toxin (ricin) content. The toxic protein ricin consists of two subunits, one a lectin that provides the uptake mechanism for the second subunit, an N-glycosidase that attacks rRNA and disrupts protein synthesis. When injected, ricin is toxic at levels as low as 0.1 microgram/kg body weight, and is one of the most toxic natural substances known. It is present in castor bean at levels up to 20 mg/g seed weight. A breeding program at Texas Tech has developed castor lines with ricin levels approaching 0.5 mg/g (4). However, the ricin and allergens remain at unacceptably high levels.

Workers who handle castor or castor meal are subject to severe immune reactions, including debilitating hives and asthma. The CB-1A fraction of castor bean extract was identified in 1943 as the source of the potent allergen present in castor bean. Recently, the 2S albumin that is part of the CB-1A fraction was identified as the major allergen of castor bean, with 96% of hypersensitive patients having IgE that binds the protein (5). The 2S albumin is a glycoprotein composed of two dimeric proteins that are derived from the same pre-cursor protein. The cDNA for the 2S albumin has been cloned and sequenced (6).

The ability to breed castor with much lower levels of these components is limited by the available germplasm. Since the allergens and toxin are proteins, the availability of molecular breeding tools make the elimination of these components feasible. We are thus evaluating the possibility of developing castor as a crop, in order to provide a domestic source of castor oil. To this end, we have developed a technique for genetic transformation. The implementation of a molecular breeding approach will provide significant reduction of both harmful components. For example, antisense gene technology has been successful in reducing expression of specific proteins by greater than 99%. Development of a suitable castor crop will provide a domestic source of castor oil, a key source of bio-based chemicals for industry.

References

1. Caupin, H.J. (1997) Products from castor oil: past, present, and future. *in* Frank D. Gunstone and Fred B. Padley (eds.) *Lipid Technologies and Applications*. Marcel Dekker, Inc. New York pp. 787-795.
2. McKeon, T.A., Lin, J.T. and Stafford, A.E. (1999) Proceedings of the 48th Oilseed Conference, New Orleans, LA. pp. H-1 - H-9.
3. Lin, J.T., Woodruff, C.L., Lagouche, O.J., McKeon, T.A., Stafford, A.E. Goodrich-Tanrikulu, M., Singleton, J.A. and Haney, C.A. (1998) *Lipids* 33, 59-69.
4. Pinkerton, S.D., Rolfe, R., Auld, D.L., Ghetie, V. and Lauterbach, B.F. (1999) *Crop Science* 39: 353-357.
5. Bashir, M.E.H., Hubatsch, I., Leinenbach, H.P., Zeppezauer, M., Panzani, R.C. and Hussein, I.H. (1998) *Int. Arch. Allergy Immunol.* 115: 73-82.
6. Irwin and Lord. (1990) *Nucleic Acids Res* 18: 5890.

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

A Simple Method for the Determination of Free Fatty Acid Content in the Oil of Fuzzy Cottonseed

Peter J. Wan¹, David R. Pakarinen¹ and D. W. Bell²

**¹Southern Regional Research Center, ARS, USDA
New Orleans, Louisiana;**

²Chickasha of Georgia, Tifton, Georgia



A Simple Method for the Determination of Free Fatty Acid Content in the Oil of Fuzzy Cottonseed

Peter J. Wan¹, David R. Pakarinen¹ and D. W. Bell²

¹Southern Regional Research Center, ARS, USDA, New Orleans, Louisiana

²Chickasha of Georgia, Tifton, Georgia

Abstract

Free fatty acid (FFA) content in the oil of fuzzy cottonseed is an important quality indicator for planting seed and cottonseed industry. Determination of FFA is a two step process, first to extract the oil from seed and followed by a quantitation procedure for FFA in the extracted oil. Official method often requires an organic solvent for oil extraction and the procedure will take a minimum of 4.5 hours to complete. A mechanical press procedure has been used by the seed breeding professionals for years. The same procedure may be useful to manage the seed in a cottonseed mill. Comparison between the 4-hr Soxhlet extraction and a mechanical press method was conducted. Each method is proven reproducible. The correlation between the two methods is also very good ($R^2 = 0.996$). However, the difference in FFA determined by the two procedures is large. Therefore, the lab who chooses to use the simple mechanical procedure to extract oil should always verify their procedure against the official method or 4-hr Soxhlet extraction. Other means to determine the amount of FFA present in the extracted oil such as the frying shortening test paper provided by 3M and a conductivity meter were examined.

Introduction

Free fatty acid (FFA) is an important quality factor in the planting seed business and oilseed industry. To insure optimum germination, cottonseed used for planting purpose should not exceed 1% FFA. The Trading Rules of National Cottonseed Products Association define a Prime Quality of cottonseed should have less than 1.8% FFA (1). Determination of FFA concentration in oilseeds is a two-step process. First the oil in the oilseed has to be extracted by either physical or chemical methods. Following extraction, FFA concentration is normally determined by titration in an appropriate solvent system. As indicated by early study that extraction method affects the amount of FFA in the oil obtained (2,3). The extraction method that takes more oil out of the oilseed generally shows higher amount of FFA. Therefore, a 4-hr Soxhlet extraction was recommended to replace the official method AOCS Aa 6-38 (4) following a collaborative study (5). Extraction has been the bottleneck for the FFA determination procedure. Therefore, development of a quick and accurate method to remove oil from oilseed has always been a high priority for the oilseed industry and instrument manufacturers. In recent years, a few instruments have been designed to satisfy the need of a rapid extraction device, such as, Soxtec, microwave assisted extractor, Accelerated Extraction System, etc. However, these are quite expensive and not mobile enough to be brought to the field or to the various seed houses. A simple method is highly desirable to the cottonseed crushing industry so that their

management of the seed quality can be carried out cost effectively. Using mechanical press to obtain oil from whole cottonseed for the FFA determination has been used by cottonseed breeders for years but few published information is available. This work is attempted to define a proper procedure for a mechanical press to be used by the cottonseed industry and examine a couple of FFA determination methods other than titration.

MATERIALS AND METHODS

Samples. Whole (fuzzy) cottonseed samples were obtained from various seed storage facilities of Chickasha Oil Mill.during 1998 and 1999.

Sample preparation Appropriate quantity of white cottonseed which was sufficiently mixed in a seed mixer which was designed and built by Mr. Bell, Chickasha Oil Mill, Tifton, GA. The same batch fuzzy cottonseed batch was dehulled in a Waring blender. Meats were recovered with a # 7 mesh sieve and ground in a Model G-3 Bunn Coffee Grinder (Springfield, IL) operated at the "drip" setting.

Oil Extraction For mechanical expression, 80 g of whole (fuzzy) cottonseed was placed in a closed-bottom cylinder (1.5" diameter) with an oil drainage tube located near the cylinder bottom. A plunger was compressed into the cylinder slowly until a final pressure of 5,000 psi following the protocol as described as follows:

1. A cotton seed sample, of .5 gallons to a maximum of 3 gallons, is mixed for a minimum of (2) minutes. The mixer turns at a rate of 20 RPM's.
2. After mixing, an 80 gram sample is removed from the mixer. In this case (3) 80 gram samples were removed and weighed to within 0.1 gram.
3. The extraction cylinder is filled, piston installed and compressed to 1000 PSI. This is done with the piston turned up side down or with the "O" ring end up.
4. Take the pressure off and remove the piston. Fill the cylinder with the remainder of the 80 grams of cotton seed. Install piston with the "O" ring end in the cylinder first.
5. Bring the hydraulic pressure to 3000 PSI and hold this pressure for 2 minutes allowing the oil to start accumulating in a vial of approx. 10 ml.
6. Increase pressure to 3500 PSI and hold for 2 minutes.
7. Increase pressure to 4000 PSI and hold for 2 minutes.
8. Increase pressure to 4500 PSI and hold for 2 minutes.
9. Increase pressure to 5000 PSI and hold for 2 minutes.

10. Release the pressure and decant oil from the cylinder drain port into the sample vial.
11. Extract the residue from the cylinder and weigh the residue. Repeat this process (3) times, the residue should not vary in weight more than 0.2 gram.

The 4-hr Soxhlet extraction of the dehulled and ground meats was done with petroleum ether.

FFA determination. The following three different methods were used for the FFA determination in the oil obtained: (1) By titration: When there was enough oil sample, the recommended official procedure was followed: 7.6 g oil was weighed and dispersed in isopropanol (75 mL) and hexane (15 mL) followed by titration against 0.25 N NaOH as described in AOCS Aa 6-38 was followed. When the oil sample was limiting, then small amount of oil was weighed and titrated with 0.1 N NaOH. All titrations were stopped at the pink end point of phenolphthalein. Final FFA percentage was calculated with the assumption that all the acids are oleic acid. (2) By using fry shortening indicator strip developed and marketed by 3M. The strip has 4 separate chemical bands. Each band, when react with oil, may turn to bright yellow from its original purple color if the FFA in the oil exceeds a pre-defined concentration. And (3) By Foodoil Sensor Model NI-21B, a conductivity meter, manufactured by Northern Technologies International Corp. (Lino Lakes, MN).

RESULTS AND DISCUSSION

FFA Determination: After repeated testing of the Foodoil Sensor and fry shortening indicator strip with a series oil samples containing known amount of free fatty acid. The sensitivity and detecting range for FFA by Foodoil Sensor is limited and was quickly determined not suitable for the FFA determination. The fry shortening indicator strip, on the other hand, demonstrated an acceptable agreement with the results obtained by titration method (Table 1). This fry shortening indicator strip was originally designed for the end point of a frying oil used by fast food service. But with some practice, it can be used as a quick estimation of the amount of FFA present in the cottonseed oil sample. It only requires 1 or 2 drops of oil for each of the four bands on the indicator strip.

Performance of Mechanical Press Method: When the mechanical press is applied following a consistent protocol as described above. The reproducibility oil yield and FFA can be better than $\pm 5\%$ of the mean. To establish the correlation between the FFA determined by titration in the oil obtained by mechanical or hydraulic press versus those by 4-hr Soxhlet extraction from the same set of six white cottonseed samples, the procedures described in the Methods Section were followed and repeated. Their mean values were plotted in Figure 1. The linear regression showed a slope of 0.64 and intercept of -1.27 with a correlation coefficient of 0.996. This correlation can be expressed in the following equation:

FFA in hydraulic pressed oil (%) = 0.64 x (FFA,%, in 4-hr Soxhlet extracted Oil) - 1.27

From this observation, it can be concluded that FFA in the oil sample obtained from mechanical method is far from parity to the expected value in the oil derived from 4-hr Soxhlet extraction. However, the mechanical method is simple well reproducible for a quick assessment of the FFA in the cottonseed that is being stored and monitored for future crushing. It is mobile and can be used in the field. With the fry shortening indicator strip, the mechanical press method can certainly be a cost efficient tool for the cottonseed oil mills to manage their cottonseed quality activities.

REFERENCES

1. *Trading Rules*, National Cottonseed Products Association Incorporated, Memphis, TN, p. 50-54, (1998).
2. Wan, P. J., D. R. Pakarinen, and P. J. Wakelyn, Concerns for the Determination of Free Fatty Acid in Cottonseed. *J. Am. Oil Chem. Soc.* **75**(10):1321-1324 (1998).
3. Wan, P. J. and Dowd, M. K. Comparative study of the extraction and measurement of cottonseed free fatty acids. *J. Am. Oil Chem. Soc.* **77**(1):23-27. 2000.
4. Official Methods and Recommended Practices of the Am. Oil Chem. Soc., 4th Ed., Third Printing, Am. Oil Chem. Soc., Champaign, IL (1993).
5. Wan, P. J. and Britton, D. A collaborative study for free fatty acid determination in cottonseed. 48th Oilseed Conference Proceedings. 18 p. 1999.

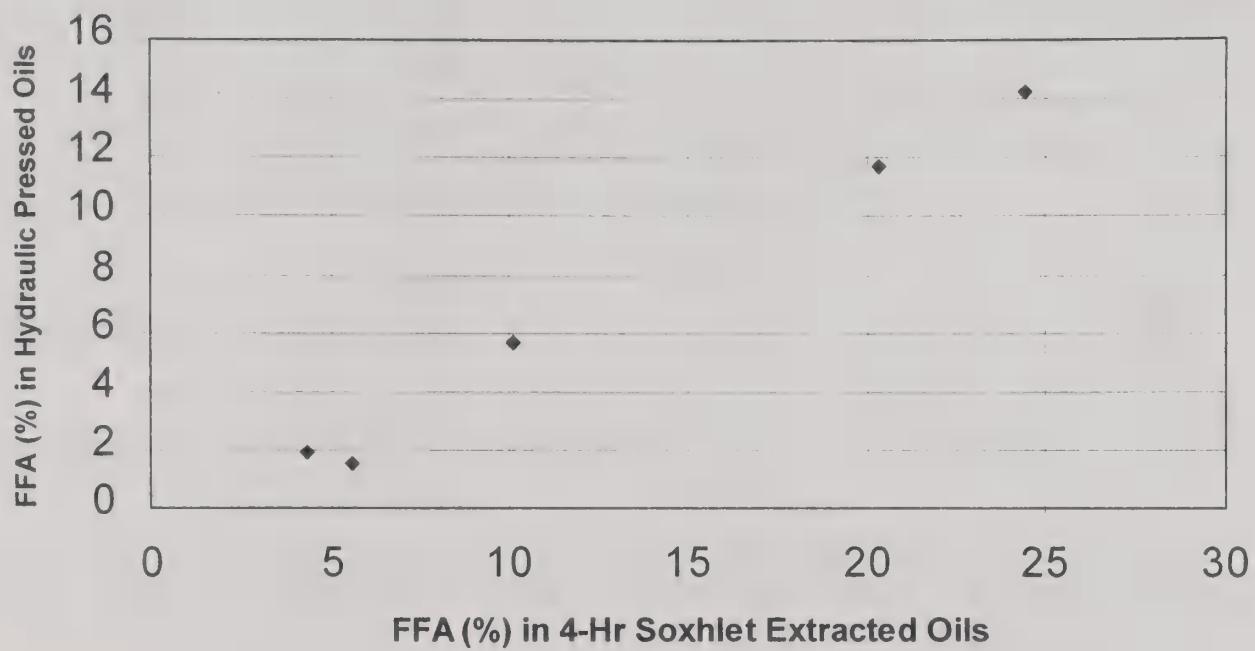
ACKNOWLEDGMENT

The authors thank the NCPA member companies for supplying cottonseed samples and Cotton, Inc. and the NCPA for financial support and many useful discussions.

Table 1. Free Fatty Acid Concentration (FFA, %) in Cottonseed Oils Determined by Titration versus Fry Shortening Test Strip

Sample Number	FFA, % By titration	FFA, % By fry shortening test strip
1	1.76	Over 1 but less than 3
2	0.50	Less than 1
3	0.92	About 1
4	5.04	About 5
5	2.70	Less than 3
6	2.58	Above 2
7	6.10	Over 5
8	7.34	Over 5

Figure 1. Free Fatty Acid (FFA) in Hydraulic Pressed vs. 4-Hr Soxhlet Extracted Oils Cottonseed Oils



A Simple Method for the Determination
of Free Fatty Acid Content
in the Oil of Fuzzy Cottonseed

Peter J. Wan¹, David R. Pakarinen¹ and D. W. Bell²

**¹Southern Regional Research Center, ARS, USDA
New Orleans, Louisiana;**

²Chickasha of Georgia, Tifton, Georgia

49th Oilseed Conference

Surviving in a Changing Global Economy

March 19–21, 2000 • DoubleTree Hotel • New Orleans, Louisiana, USA

Bioconversion of Fats and Oils into Value-Added Products

William N. Marmerⁱ, Thomas A. Foglia,
Daniel K.Y. Solaiman, and John P. Cherry
USDA, ARS, Eastern Regional Research Center
Wyndmoor, PA



Bioconversion of Fats and Oils into Value-Added Products

William N. Marmer¹, Thomas A. Foglia, Daniel K. Y. Solaiman, and John P. Cherry

USDA, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038

Expanding on a rich history of fats and oils research at ERRC,² two current projects are at the forefront of promoting the conversion of fats and oils commodities into value-added products using biotechnology. Both projects address the goals of ARS National Program 306, "New Uses, Quality, and Marketability of Plant and Animal Products."³ The biodiesel component of one project also addresses ARS National Program 307, "Bioenergy and Energy Alternatives."⁴

"New Processes for Obtaining Higher Value-Added Products from Agricultural Lipids"

This project (CRIS Project #1935-41000-048) is led by Dr. Thomas A. Foglia, and includes in its senior staff Drs. Michael J. Haas, An-Fei Hsu, William N. Marmer, Alberto Nuñez, and George J. Piazza. New processing technologies based on biocatalysis and/or biomimetic reagents are being developed for the conversion of agriculturally-derived triglycerides -- animal fats and vegetable oils -- into higher value-added products. Targeted research thrusts include the lipase-catalyzed harvesting of industrially important fatty acids from fats and oils; the enzymatic production of industrial biopolymers, and the enzymatic or biomimetic production of oxygenated and branched-chain fatty acids from agrochemical glycerides. Much of the research invokes the mild conditions and structural selectivity of selected biocatalysts from a "toolbox" of lipases and other enzymes. Specific objectives include:

Immobilized enzymes and their use as biocatalysts:

We are investigating newer methods to immobilize enzymes using several related entrapment techniques, namely sol-gels and phyllosilicate clays cross-linked with sol-gels. Several profitable lines of research are being conducted with natural fats and oils using these unique enzyme preparations, such as the harvesting of industrially important fatty acids and the continuous oxygenation of unsaturated fatty acids. Other work focuses on determining the thermal stability and reusability of these intercalated immobilized enzymes in continuous

bioreactors. Targeted enzymes are lipases and lipoxygenases. The main advantages: excellent enzyme activity and retention of activity through numerous recyclings.

Enzymatic restructuring of natural glycerides to nutraceutical lipids:

A key to developing lipids with new performance properties is to discriminate between fatty acids that differ in size or degree of unsaturation. By replacing chemical catalysts with enzymes, we have developed new ways of synthesizing triacylglycerols (TAG) with specific fatty acid compositions and arrangements, and thus specific applications. Such “structured lipids” are presently entering the marketplace, and additional new structured lipids are envisioned. Among these structured lipids are so-called nutraceuticals, marketed as nutritional supplements. Some of our research in this area is in partnership with industry.

Enzymatic harvesting of valuable fatty acids from commodity fats and oils:

Examples of success include obtaining 13 c -22:1 (erucic acid) from HEAR oil, 6 c ,9 c ,12 c -18:3 (γ -linolenic acid) from borage, 9 c ,10 t -C18:2 (conjugated linoleic acid, CLA), 6 c -18:1 (petroselinic acid) from coriander, C20/C22 unsaturates from meadowfoam, and 9 c -12-(OH)-18:1 (ricinoleic acid) from castor.

Enzymatic production of industrial biopolymers:

We are investigating the use of lipases for the synthesis of polymers from natural, agriculturally derived materials. Targeted areas of research include the synthesis of polyesters from glycerol and fatty dicarboxylic acids, and fatty acylated biopolymers from oligomeric sugars. The challenge of this work is to change the hydrophobic-hydrophilic balance of carbohydrates to improve or expand the thixotropic properties of natural carbohydrate oligomers and polymers. Targeted applications include plasticizers, thickeners, and films. Other work on production of biopolymers from fats and oils is incorporated into the second fats and oils project at ERRC (*cf.* below).

Production of oxygenated fatty acid derivatives:

The objectives of this work are the production of epoxidized fats and oils for use as plasticizers and other industrial oxygenated products. The approaches use enzymes as catalysts and organic

hydroperoxides as the oxidant. Current challenges include the epoxidation of intact fats and oils and the use of the less expensive hydrogen peroxide as the oxidant. Other work studies the enzymatic conversion of hydroperoxy fatty acids, produced by a previously developed technology, into dicarboxylic acids for the production of higher nylons and polyesters. Other materials readily obtainable from epoxy and peroxy fats and oils include polyhydroxy and epoxy-hydroxy fatty acids, potential plastizers, emulsifiers and detergents. Recently, we have begun to explore the use of peroxygenases to effect epoxidation of unsaturated fatty acids.

Reactions of enzymes in supercritical fluids:

We are investigating the reaction of enzymes in supercritical fluids to develop processes for the simultaneous reaction and processing of lipid and restructured lipid materials. Additional advantages of this new technology include retention of enzyme activity for reuse, no solvent usage, and milder reaction conditions for isolation of heat-sensitive and oxidation-prone fatty acids (PUFA).

Biomimicry as applied to fatty acid chain modification.

We are investigating a number of newly developed biomimetic processes for introducing useful chemical functionality into the hydrocarbon chains of fatty acids. Many of these derivatized fatty acids are already used by industry but more efficient processes are needed to exploit the full potential of these novel materials. Included in these studies are more efficient catalytic procedures for producing branched-chain fatty acids from their normal chain counterparts.

Biodegradable fuels, fuel additives and lubricants:

Our research on biodiesel strives to improve the economics of production by incorporating cheaper feedstocks--tallow, greases and soapstock. Cold temperature properties of the biodiesel are improved by preparing ethyl or isopropyl esters in place of the traditional methyl esters. Differences in cold-temperature properties are even less dramatic when biodiesel is blended with soy biodiesel and when that biodiesel blend in turn is blended 20:80 (v/v; so-called B20) with petrodiesel. Cold temperature properties aside, the more saturated the biodiesel, the lower the NO_x emissions from its burning. Other studies concern the establishment of quality and storage stability standards for biodiesel fuels. Still other interests center on the production of

biodegradable fuel and lubricant additives from fats and oils. Targeted areas include oxygenated additives as cetane number improvers, lubricity agents, and hydraulic fluids.

"Bioconversion of Agricultural Fat, Oil and Derivatives into Value-Added Biopolymers"

This project (CRIS Project #1935-41000-050) is led by Dr. Daniel K. Y. Solaiman, and includes in its senior staff Drs. Richard Ashby, Thomas A. Foglia, and Cheng-Kung Liu. Its goal is to develop cost-effective processes for the production of biodegradable polymers and surfactants from agricultural fats and oils. Specific goals include: (1) identification of microbial strains capable of biotransformation of triglyceride feedstocks into biodegradable polymers and surfactants; (2) genetic manipulation of these strains to achieve high-level synthesis; (3) development of a high-yield fermentation process; (4) *in vitro* modification and characterization of the products to target them for commercial applications. Specific objectives include:

Strain improvement of lipid-metabolizing organisms that produce poly(β -hydroxyalkanoate) polyesters (PHA) or sophorolipid emulsifiers (SL):

We are screening for wild-type high PHA-producing microorganisms capable of using intact fats and oils as growth substrates. To facilitate the effort, we have developed a polymerase-chain-reaction (PCR) method for screening PHA synthase-encoding genes in pseudomonads. We also are using a genetic engineering approach to endow known PHA-producing bacteria with fat and oil-metabolizing gene(s), and *vice versa*. To this end, we have developed a genetic transfer system based on electroporation to allow for the transformation of pseudomonads. We have analyzed and determined the nucleic-acid sequence of a region of *Pseudomonas resinovorans* chromosome coding for the PHA metabolism functions. Based on the sequence information, the individual genes (e.g., PHA synthases and PHA depolymerase) have been cloned by PCR. We are now using these genes to develop high-expression transformants to modulate polymer yields and to genetically engineer chimeric enzymes that have altered activity and specificity. Our lab is presently investigating the use of mildly thermophilic bacteria, e.g., *Bacillus thermoleovorans*, as producer strains to facilitate the utilization of high-titer fats and oils, such as tallow and lard.

Manipulation of the properties of bio-based products by varying the feedstocks:

We have demonstrated that the chemical composition and physical characteristics of PHA vary according to the type of lipid feedstock. For example, soybean oil-derived PHA contains pendant groups with unsaturated carbon bonds and is highly amorphous. In contrast, tallow-derived PHA is elastomeric and contains highly saturated side chains. We are expanding the range of the fat and oil substrates to include restaurant grease and oils from experimental plants, such as *Lesquerella*. The use of highly substituted substrates should produce functionalized PHA useful for further derivatization and modification. In a collaborative effort, similar strategies are being explored for the manipulation of SL composition and properties.

Development of environmentally friendly purification processes:

We are exploring the use of supercritical fluid extraction (SFE) as a means to isolate and purify PHA. Our earlier results indicated that SFE of the harvested cells improves the purity of the PHA subsequently extracted with organic solvents. We are applying this SFE procedure to purify bulk-produced, lower grade PHA for use in biomedical applications requiring high-purity materials. This approach will be especially attractive to purify plant-derived PHA. Purification of SL involves the use of organic solvents, such as hexane and ethyl acetate. We are examining the use of SFE in the purification of SL's.

Post-production modification of bio-based products:

Our preliminary results showed that the physical properties of PHA may be changed by epoxidation and/or crosslinking (autoxidation; UV- or γ -irradiation) to suit a specific application. We are continuing to improve the efficiency of these reactions and to explore other chemical functionalization reactions of PHA and SL.

Materials processing of PHA:

We have experimented with the solution-casting of various PHA's into films of different strength and elasticity. Industry is testing our tallow-derived PHA for adhesive properties. Additional collaboration will allow us to test the suitability of selected PHA's for spinning into fibers.

In conclusion, our research program on fats and oils utilization progresses within the guidelines of the research program of the Agricultural Research Service. Like other fats and oils-related research in this Agency, ours is goal-oriented and targets the adding of value to domestic commodities, and it is also long-range and risky in nature and national in scope. Although the biotechnological flavor of the current research is markedly different from earlier times, our goal is to maintain the excellent record of accomplishment and impact of those days. We welcome your contact and we invite you to browse our website.⁵

¹ Contact information: telephone 215-233-6585; e-mail wmarmer@arserrc.gov.

² Marmer, W. N., Fattening the market value of vegetable oils: A long-term and successful USDA mission. 48th Oilseed Conference, New Orleans, LA, March 1999.

³ See <http://www.nps.ars.usda.gov/programs/306s2.htm>.

⁴ See <http://www.nps.ars.usda.gov/programs/307s2.htm>.

⁵ See <http://www.arserrc.gov>; select "Research;" Select "Hides, Lipids and Wool;" select the appropriate "Research Area," and then browse through the lists of subtopics within the Research Area. The homepage includes a searchable bibliography.